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Two-Dimensional Movement Patterns of Juvenile Winter-Run and Late-Fall-Run Chinook Salmon at the Fremont Weir, Sacramento River, CA

Anna E. Steel, Bertrand Lemasson, David L. Smith,
and Joshua A. Israel

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Two-Dimensional Movement Patterns of Juvenile Winter-Run and Late-Fall-Run Chinook Salmon at the Fremont Weir, Sacramento River, CA

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Abstract

To improve modeling of juvenile salmon behavior and movement in the Sacramento River, smaller winter-run Chinook and larger late-fall-run Chinook salmon were tagged and released into a 2D telemetry array during the winter of 2015. Detection positions were filtered and discretized to create two-dimensional tracks and measure movement characteristics, evaluate space use, and assess whether these runs displayed distinct behavioral differences. Speed over ground and turning angle were not significantly different between release times, fish size, or run. Only the initial movement rate between release and array locations was significantly different between the runs. Both runs displayed a non-uniform distribution within the channel and tended to use space along the outer bend more frequently than the inner bend. Winter-run Chinook salmon tracks were slightly farther towards the outer bend than late-fall-run Chinook. A similar result was not observed in smaller and larger late-fall-run Chinook, which suggested that differential space use may be influenced more by run identity than variation in size between runs. Although small differences between runs were measured, it is reasonable to aggregate these results for a singular juvenile salmon behavior model, rather than developing independent juvenile behavior models based on adult run-timing.

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Preface

This study was conducted for and funded by the US Bureau of Reclamation under Project Number R13PB20203, “Habitat Restoration and Fish Passage Research and Evaluation.” The technical monitor was Dr. Pat Deliman, Technical Director, Environmental Engineering and Science.

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At the time of publication, Mark Noel was Acting Chief, CEERD-EPW; Warren P. Lorentz was Chief, CEERD-EP; and Dr. Pat Deliman, CEERD-EZT, was the Technical Director for Environmental Engineering and Science. The Deputy Director of ERDC-EL was Dr. Jack E. Davis and the Director was Dr. Beth C. Fleming.

COL Bryan S. Green was the Commander of ERDC, and Dr. David W. Pittman was the Director of ERDC.

Unit Conversion Factors

Multiply	By	To Obtain
acres	4,046.873	square meters
acre-feet	1,233.5	cubic meters
cubic feet	0.02831685	cubic meters
degrees (angle)	0.01745329	Radians
degrees Fahrenheit	$(F-32)/1.8$	degrees Celsius
feet	0.3048	Meters
hectares	1.0 E+04	square meters
miles (US statute)	1,609.347	Meters
miles per hour	0.44704	meters per second
ounces (mass)	0.02834952	Kilograms
ounces (US fluid)	2.957353 E-05	cubic meters
pints (US liquid)	0.473176	Liters

1 Introduction

1.1 Background

On June 4, 2009, the National Marine Fisheries Service (NMFS) issued its final Biological Opinion and Conference Opinion on the Long-Term Operation of the Central Valley Project (CVP) and State Water Project (SWP) (NMFS Operation BO). The NMFS Operation BO concluded that, if left unchanged, CVP and SWP operations were likely to jeopardize the continued existence of four federally listed anadromous fish species: Sacramento River winter-run Chinook salmon, Central Valley spring-run Chinook salmon, Central Valley steelhead (*O. mykiss*), and Southern Distinct Population Segment (DPS) North American green sturgeon (*Acipenser medirostris*). The NMFS Operation BO sets forth Reasonable and Prudent Alternative (RPA) actions that would allow continuing SWP and CVP operations to remain in compliance with the federal Endangered Species Act (ESA). These include restoration of floodplain rearing habitat, through a “notched” channel that increases seasonal inundation within the lower Sacramento River Basin. A significant component of these risk reduction actions is lowering a section of the Fremont Weir (Figure 1) to allow juvenile fish to enter the bypass and to allow adult fish to more easily ascend this hazard. Questions remain on the details of notch implementation (size, location), fish entrainment efficiency, and species-specific and ontology-based behaviors.

1.2 Objective

The purpose of this study is to quantify the behavior of winter-run Chinook and late-fall-run Chinook (*Oncorhynchus tshawytscha*) in the Sacramento River adjacent to the Fremont Weir. This weir serves as the boundary between the main channel of the river and the Yolo Bypass (Bypass), a major flood bypass reach.

Results from this study will be used to inform a fish behavioral model using the Eulerian–Lagrangian–Agent Method (ELAM) to predict fish behavior in the region for floodplain restoration planning. Floodplain restoration is needed because there have been significant modifications made to the historic floodplain of California’s Central Valley for flood damage reduction purposes and water supplies. The resulting losses of

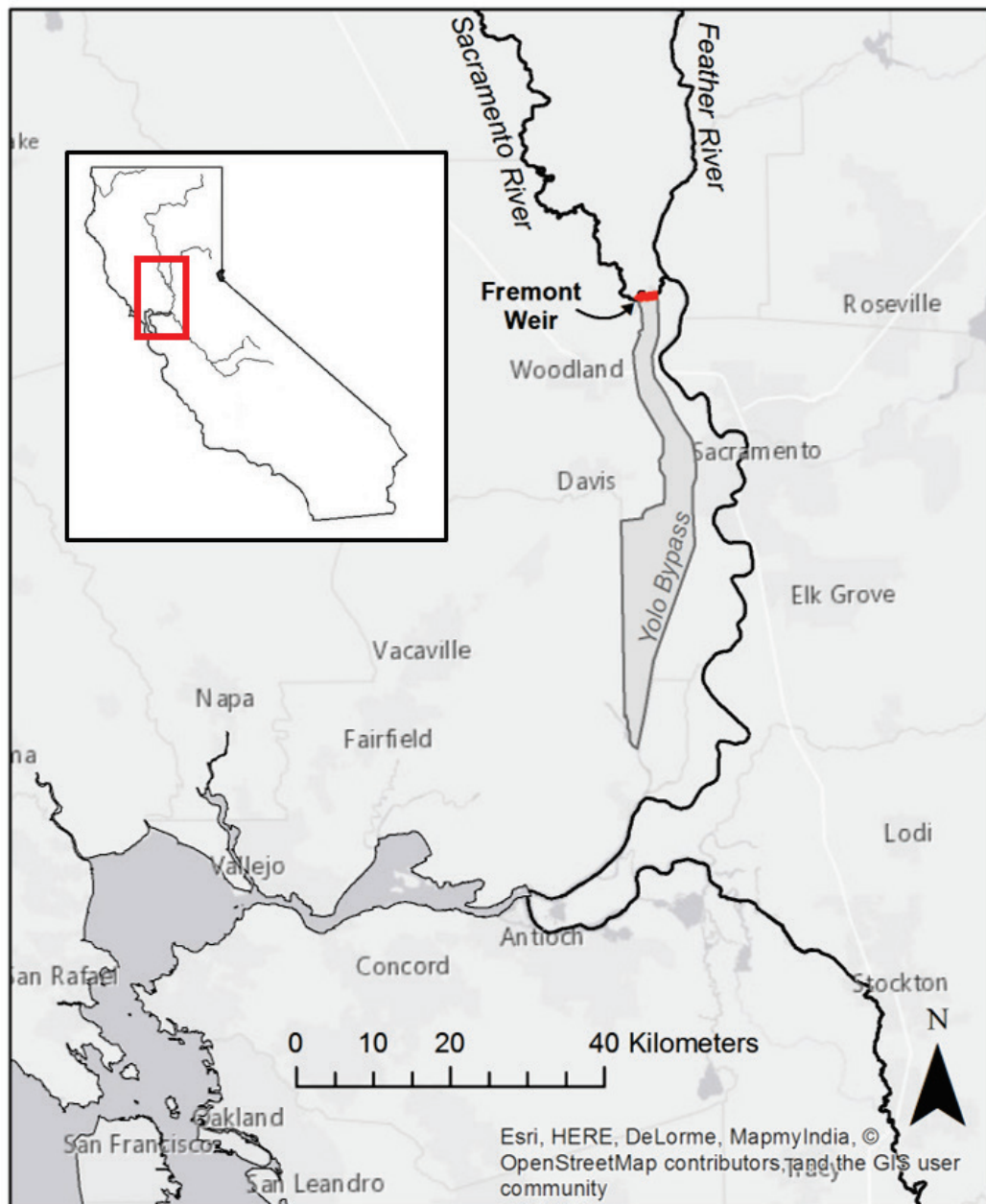
rearing habitat, migration corridors, and food web production for fish have significantly hindered native fish species that rely on floodplain habitat during part or all of their life history. The Bypass, which currently experiences at least some flooding in approximately 80% of years, still retains many characteristics of the historic floodplain habitat that are favorable to various fish species. In approximately 70% of years, Fremont Weir overtops, connecting the Bypass to the Sacramento River along its northern boundary, and Sacramento flows join flows from western tributaries. In approximately 10% of years, localized flooding is due to western tributary contributions only. The primary function of the Bypass is flood damage reduction, with most of it also managed as agricultural land. The Bypass has also been identified by several state and federal entities as a potential site for habitat restoration to ease pressure on and increase benefits to threatened and endangered fish species.

1.3 Approach

The system of weirs on the Sacramento River was designed with the understanding that runoff from many of the storm events experienced in the Sacramento River watershed cannot be contained within the banks of the river. Nor could this flow be fully contained within a levee system without periodically flooding adjacent property. Thus, the weirs were designed to occasionally spill through a system of weirs and flood relief structures into adjacent basins. These basins are designed to contain flood waters and channel them downstream to eventually be conveyed back into the Sacramento River near Knights Landing and Rio Vista. Dry weather flows are contained within levees near the river banks, and land within the flood basins is then used for agricultural purposes.

Fremont Weir is one weir on the Sacramento River (Figure 1). It was completed in 1924 by the USACE. It is the first overflow structure on the river's right bank and its two-mile overall length marks the beginning of the Yolo Bypass. It is located about 15 miles northwest of Sacramento and eight miles northeast of Woodland. South of this latitude, the Yolo Bypass conveys 80% of the system's floodwaters through Yolo and Solano Counties until it connects to the Sacramento River a few miles upstream of Rio Vista. The weir's primary purpose is to release overflow waters of the Sacramento River, Sutter Bypass, and the Feather River into the Yolo Bypass. The crest elevation is approximately 33.5 ft (USED) and the project design capacity of the weir is 343,000 cfs. Adding the notch to this weir will change the amount of time that water flows over it and increase access to the floodplain for juvenile salmon.

Figure 1. Map of the lower Sacramento River, California, indicating the Yolo Bypass and Fremont Weir.



2 Study Design and Data Collection

2.1 Acoustic positioning array

2.1.1 Equipment

This study used acoustic tracking technology developed by Vemco (Halifax, NS, Canada). Fish are outfitted with acoustic transmitters, or tags, that emit a series of pulses, which include a uniquely identifiable code. The signals are received by autonomous units with hydrophones and processing software that includes high-precision internal clocks. To position tagged fish in two dimensions, 40 high-resolution receivers (HR-1, 180 kHz) were deployed through the study area. HR-1 receivers were used because they can detect tags using both pulse-coding and frequency-modulated coding technologies. The latter format reduces concern about overlapping tag signals and loss of detections common with pulse-coded transmissions (Voegeli et al. 2001). When a single tag signal is detected at multiple receivers, the differences in the time of detection can be used to calculate the distance of the tag from each receiver, thus allowing its position to be calculated. For further discussion of 2D positioning see Espinoza et al. (2011), Roy et al. (2014), and Steel et al. (2014).

Because receivers are autonomous units, the clocks must be synchronized during post processing to avoid positioning errors due to clock drift. To achieve this, each receiver is deployed with a co-located sync tag, which allows for later synchronization. To provide information about the accuracy of the positioning array, reference tags are also deployed throughout the study area to estimate positioning error. In this study, 21 reference tags were placed at known locations, primarily along the center of the channel.

2.1.2 Deployment and retrieval

Receivers, sync tags, and reference tags were deployed by US Geological Survey based on the suggested positioning provided by Vemco. Equipment mounts were designed to maintain the receivers in position at an upright orientation (Figure 2). Receivers were located with hydrophone tips approximately 65 cm above the substrate. Each receiver was co-located with a sync tag, attached to the mount at approximately 112 cm above the substrate. At five of the forty mounts, the receiver and sync tags were placed an additional 56 cm above the substrate.

Figure 2. Image of a receiver as deployed within the study area.



Mounts were deployed with the assistance of divers to ensure mounts were in a stable and upright location. Two to four mounts were attached to a cable, which was then anchored to shore. When complete, the array included 14 cabled lines of receivers, which covered approximately 0.7 river km. This section of the river ranged from 60 to 100 m wide (Figure 3).

Figure 3. Forty tag-detecting receivers (Vemco) were deployed along approximately 0.7 km of the Sacramento River at the site of the Fremont Weir. The array design allowed for the fine-scale positioning of tagged juvenile salmon as they migrated through the river.

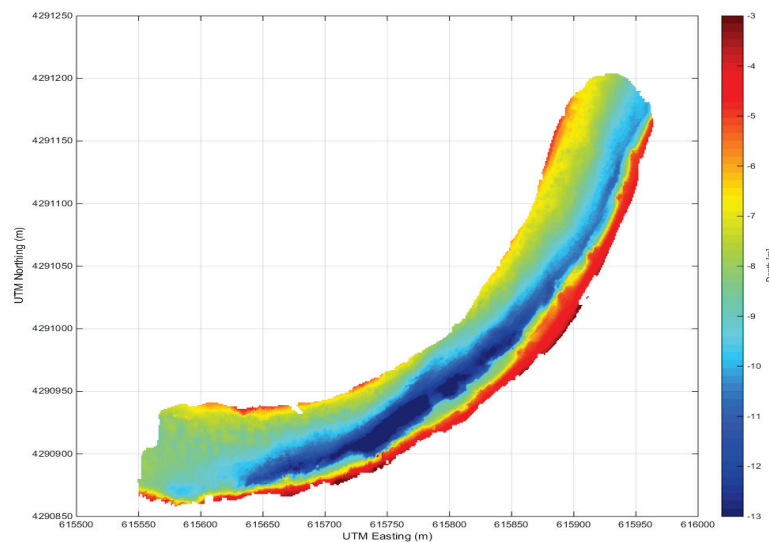


The retrieval of equipment began on 6 Feb 2015. A storm was forecast with large expected increases in river stage, which raises the likelihood of equipment becoming buried in sediment or snagged by debris, preventing retrieval. The loss of data from a few receivers would jeopardize the ability of the entire system to accurately calculate fish positions; consequently, the team removed the equipment to ensure quality data for those fish that had already passed through the array. Array removal was also conducted by US Geological Survey.

2.2 Hydraulic and bathymetry data

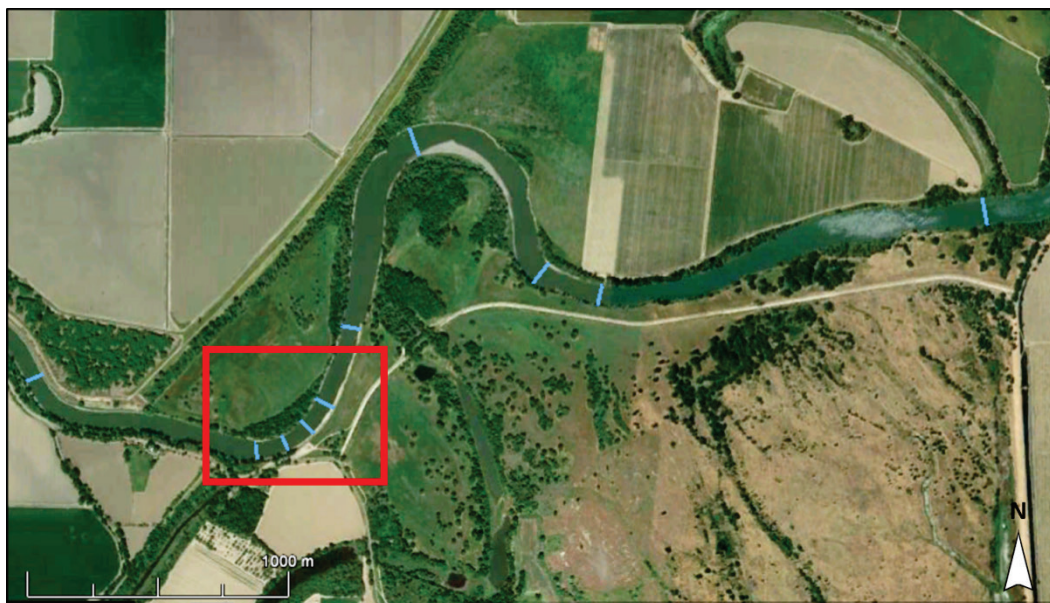
Initial surveys of the geomorphology and hydrology of the study reach were completed in November and December 2014 by the United States Geological Survey (USGS). River velocities and bathymetry were mapped from a boat with an RDI Rio Grande Workhorse acoustic Doppler current profiler (ADCP) and a differential GPS. The results from these surveys were used to create bathymetry maps for the study coordination team to complete successful planning of the study design (Figure 4). Additional extensive surveys were conducted by the California Department of Water Resources (DWR) with a Knudsen Engineering Limited Sounder 1612 survey-grade echosounder and transducer. Surveys were conducted between 21 January and 27 January 2015 to create bathymetric maps of the entire region (See Appendix H for cross-section locations). Cross-sectional sweeps from these surveys were combined with a longitudinal profile collected on 8 April 2015 to create final bathymetric maps for use in computational fluid dynamic modeling (see Lai 2016 for details).

Figure 4. Bathymetric map of local study area, from data collected by ADCP surveys.



To estimate current profiles in the study region during the period of fish releases, additional ADCP surveys were conducted by California DWR. Surveys were not conducted concurrently with fish passage windows to avoid unwanted interference between the ADCP equipment and the acoustic receivers, as noise created by ADCP equipment can reduce fish-tag detection. An initial survey was completed on 26 January 2015 just prior to the first release of fish, at a river stage of 14.5 ft. Ten cross-sectional transects were surveyed, with six separate passes at each cross-section during a single survey (Figure 5). The processed data were used to verify results from the computational fluid dynamic modelling (Lai 2016), which is used to support ELAM modeling of fish behavior in the study area.

Figure 5. Map of cross-sectional transects in Sacramento River surveyed with ADCP equipment to map hydrodynamics. Area outfitted with acoustic receivers indicated by red box.



2.3 Study fish

2.3.1 Tagging

Acoustic tags were surgically implanted in the study fish following the methods outlined in Appendices A and B. Tags were manufactured by Vemco, model V4, measuring 11 mm and 0.42 g in air. They emitted a signal at 180 kHz, and were programmed to transmit at a random interval every 1-2 sec. Surgical procedures were derived from Liedtke et al. (2012), and from procedures used by Cramer Fish Sciences, by DOI's Interior South Delta telemetric studies, and by the US Army Corps of Engineers, Sacramento District telemetric studies. A total of 499 fish were tagged and released, consisting of 249 winter-run and 250 late-fall-run juvenile Chinook (there was one winter-run mortality before release). Winter-run Chinook (WFC) were acquired, tagged, and held at Livingston Stone National Fish Hatchery, and late-fall-run Chinook (LFC) were acquired, tagged, and held at Coleman National Fish Hatchery. Due to the size of the tag and a desire to keep the tag burden less than 5%, study fish were limited to those >8.2 g at the time of tagging.

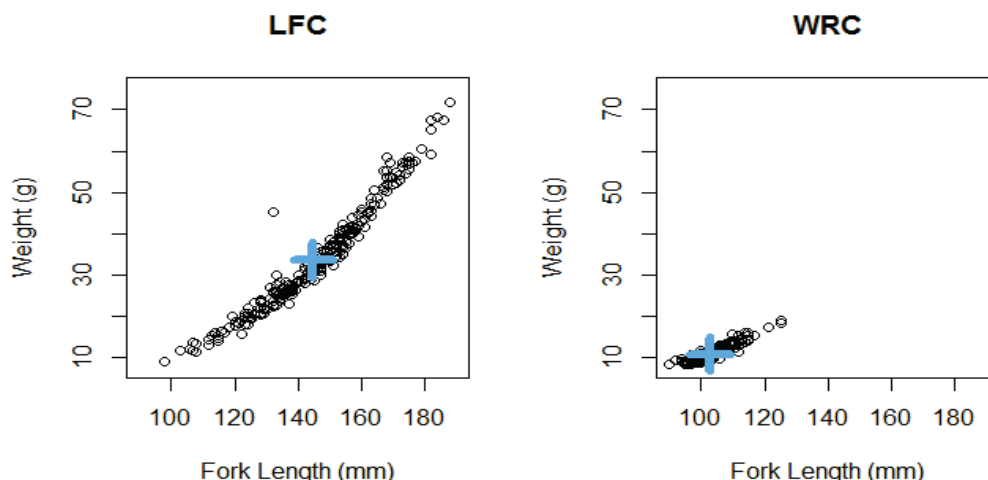
Before surgery, fish were anaesthetized individually in a 19L bucket. The water was super-saturated with oxygen, to a level of 120-150%. Dissolved

oxygen levels were maintained using an airstone and pump. Due to differing regulations for groups listed under the Endangered Species Act, different anesthetic drugs were used for the two runs. WRC were anesthetized with tricaine methanesulfonate (MS222), beginning at dosages of 70 mg L⁻¹, and buffered to a pH of 7 - 8 with sodium bicarbonate. LFC were anesthetized with AquiS®, beginning at dosages of 30mg L⁻¹. Both dosages were adjusted as needed to ensure that fish reached anesthesia within 2-4 min. Fish were rejected from the study if they were anesthetized in less than 1 or more than 5 min ($N_{LFC} = 11$, $N_{WRC}=6$). A small amount of Stress Coat® (a water conditioner and artificial slime coat) was also added to anesthetic baths to protect fish from loss of the slime layer during tagging.

Upon reaching surgical anesthesia, fish were weighed and measured (fork length, FL) and assessed on a categorical scale for condition (eyes, fins, scales). Full anesthesia was defined as loss of equilibrium and no response to firm pressure on the caudal fin (Neiffer and Stamper 2009). Any fish classified as poor were rejected from the study ($N_{LFC} = 7$, $N_{WRC}=6$). Fish were then transferred to a microcell foam surgical cradle where the gills were bathed with a maintenance dose of anesthesia (20 mg L⁻¹ MS-222; 10 mg L⁻¹ AquiS®). An incision approximately 5 mm long was made parallel to and offset from the ventral line, anterior to the pelvic girdle. A disinfected tag was placed into the peritoneal cavity of the fish and positioned to lie immediately under the incision. The incision was then closed with a simple suture, using a 3/8 circle needle with 4/0 Mono-Dox (violet monofilament polydioxanone) suture material. All surgical materials were disinfected before surgery and between fish. Any surgery that did not meet quality standards resulted in the fish not having been implanted with a tag and the specimen was allowed to recover ($N_{LFC} = 4$, $N_{WRC}=14$).

Due to differences in life-history timing, juveniles from each run were different in size during the period of tagging. Fish were selected from available stock at random, and rejected if they were below 8.2 g or greater than 200 mm fork length (FL; $N_{LFC} = 1$, $N_{WRC}=21$). The tagged LFC had a mean length of 145 mm FL, and WRC had a mean length of 103 mm FL. The mean weight of tagged LFC was 34 g, while the WRC mean weight was 11.0 g (Figure 6).

Figure 6. Comparison of the size of tagged fish used in the study. LFC = late-fall-run Chinook juveniles; WRC = winter-run Chinook juveniles. Blue crosses indicate mean size.



Post-surgery, fish were allowed to recover alone in a 19L bucket for 10 min. After confirming the fish was upright and actively swimming, it was transferred to a circular holding tank. If a fish did not recover to a state of active swimming within a reasonable period of time (>1 hr), it was euthanized and the tag was removed ($N_{\text{LFC}} = 1$). Each circular holding tank contained 25 fish with known tag IDs, with the exception of four circulars at Coleman National Fish Hatchery; each of these four tanks held 50 LFC. Fish were held in these circular tanks for 1-2 weeks, depending upon their assigned release date, and were fed daily except for the final day before release. Each day tanks were scanned for mortalities, and the water was monitored for dissolved oxygen (mg L^{-1} and % saturation) and water temperature ($^{\circ}\text{C}$). Throughout the surgical and recovery process, water temperatures in all tagging and transport containers were never greater than 2°C different from the reference water source where the fish were raised.

2.3.2 Transport and release

To transport fish from the hatchery to the release site, fish were loaded into oxygenated coolers and driven roughly 2.5 hr to a private dock near the town of Knights Landing. No more than 13 fish were transported in a single cooler, and rock salt was used to increase the salinity of the transport water to around 3 ppt. Transporting fish in mildly brackish water reduces the osmotic gradient between a fish and its environment. This is considered a good practice, as stress from handling causes fish to produce epinephrine, which, in turn, increases gill surface area (Wedemeyer

1996). The physiological change allows freshwater to diffuse inward more rapidly, and if the osmotic gradient is large, this diffusion can overwhelm the osmotic and ionic regulatory controls of the fish. Adding salt to the transport water reduces this ion imbalance and reduces stress for the fish (Moyle and Cech 2004). In addition, Stress Coat®, a water conditioner and artificial slime coat, was added to transport water to help the fish to maintain a mucus layer as a barrier against disease and infection, as handling of fish can also reduce the natural mucus layer (Harnish et al. 2011).

Water quality parameters (dissolved oxygen, water temperature, and salinity) were measured upon transfer of fish from hatchery tanks into coolers, halfway through transport, and upon arrival at the release site. Upon arrival at the release site, if there was greater than 2°C of difference in water temperature between the coolers and the river, a slow exchange of water was used to equilibrate the coolers to river temperature without shocking the tagged fish.

Once the temperatures were within 2°C of one another, fish were transferred from the coolers into in-river holding pens using sanctuary nets. Before fish were removed from coolers, a customized HR-180kHz-EXT receiver was placed in each cooler to record the tags present.

In-river holding pens were circular, made of perforated heavy-duty plastic (1/4 in. holes), approximately 120 L, and had a tight-fitting lid. All pens floated in the river alongside a dock, with air-space between the top of the water and the lid so smolts could fill their swim bladder at the surface if needed. Each pen contained the same 25 individuals, which were held together in circular tanks at the hatchery prior to transport, except for those hatchery tanks that contained 50 fish. In these cases, two in-river pens contained the individuals originally held together. Fish were habituated to the river in these pens for a minimum of 24 hr, while the team checked dissolved oxygen in the adjacent river every 4 hr to ensure fish were experiencing suitable water quality conditions.

After the initial holding period, fish from two pens constituting 25 WRC and 25 LFC were released in the center of the channel every 5 hr throughout the following 24 hr period (Figure 7). At each release, fish were allowed to volitionally swim from the pens. Water quality measurements were recorded at the time of release. When fish were released, the river stage was approximately 14.6 ft at the Fremont Weir gauge, corresponding to a discharge of approximately 5700 cfs (Table 1).

Figure 7. Release times of study fish indicated by vertical red lines, overlaid on a hydrograph of the Sacramento River near the release site. All fish experienced very similar release conditions.

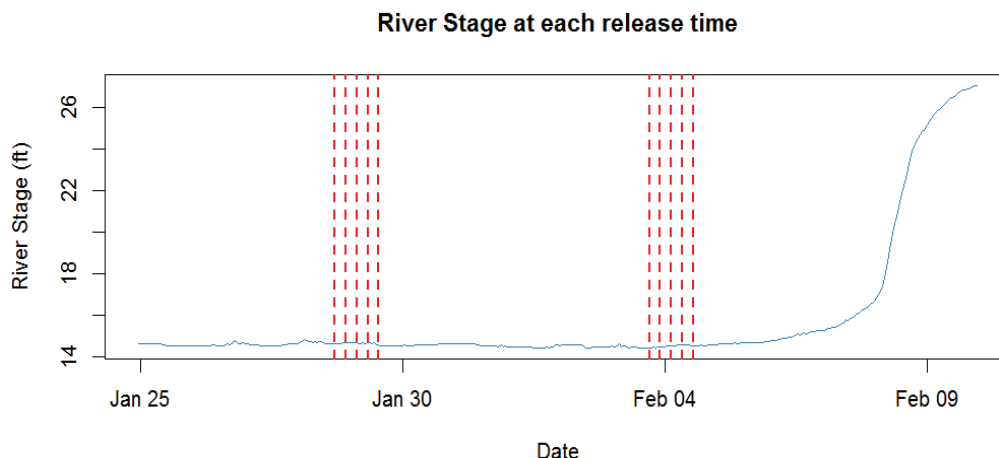


Table 1. Release groups (ca. 25 individuals from each run) were released every five hours during each 24 hr release period. The river stage, measured at Fremont Weir (FRE), and the neighboring discharge gauge 56 rkm upstream at Wilkins Slough (WLK) both suggest similar release conditions for each group. Two winter-run individuals were not released; one was a mortality before release and the second escaped during transfer to the river holding pens.

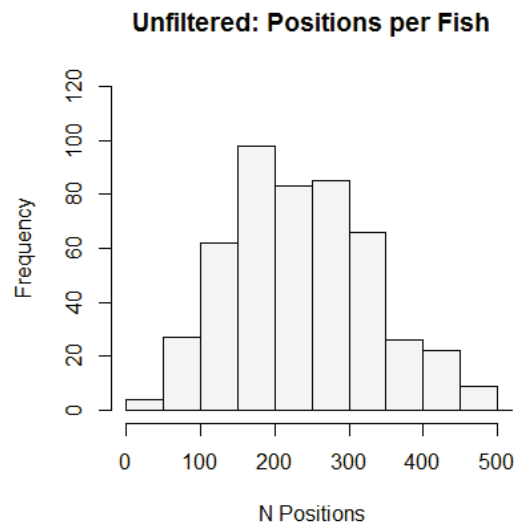
Release Date	Release Hour (PST)	Stage @ FRE (ft)	Q @ WLK (cfs)	N _{LFC}	N _{WRC}
01-29-2015	17:00	14.63	5710	25	25
01-29-2015	22:00	14.65	5710	25	24
01-29-2015	03:00	14.65	5710	25	25
01-29-2015	08:00	14.68	5700	25	25
01-29-2015	13:00	14.54	5690	25	24
02-04-2015	17:00	14.39	5600	25	25
02-04-2015	22:00	14.42	5610	25	25
02-04-2015	03:00	14.52	5600	25	25
02-04-2015	08:00	14.58	5590	25	25
02-04-2015	13:00	14.52	5630	25	25

3 Statistical Analysis and Results

3.1 Array performance

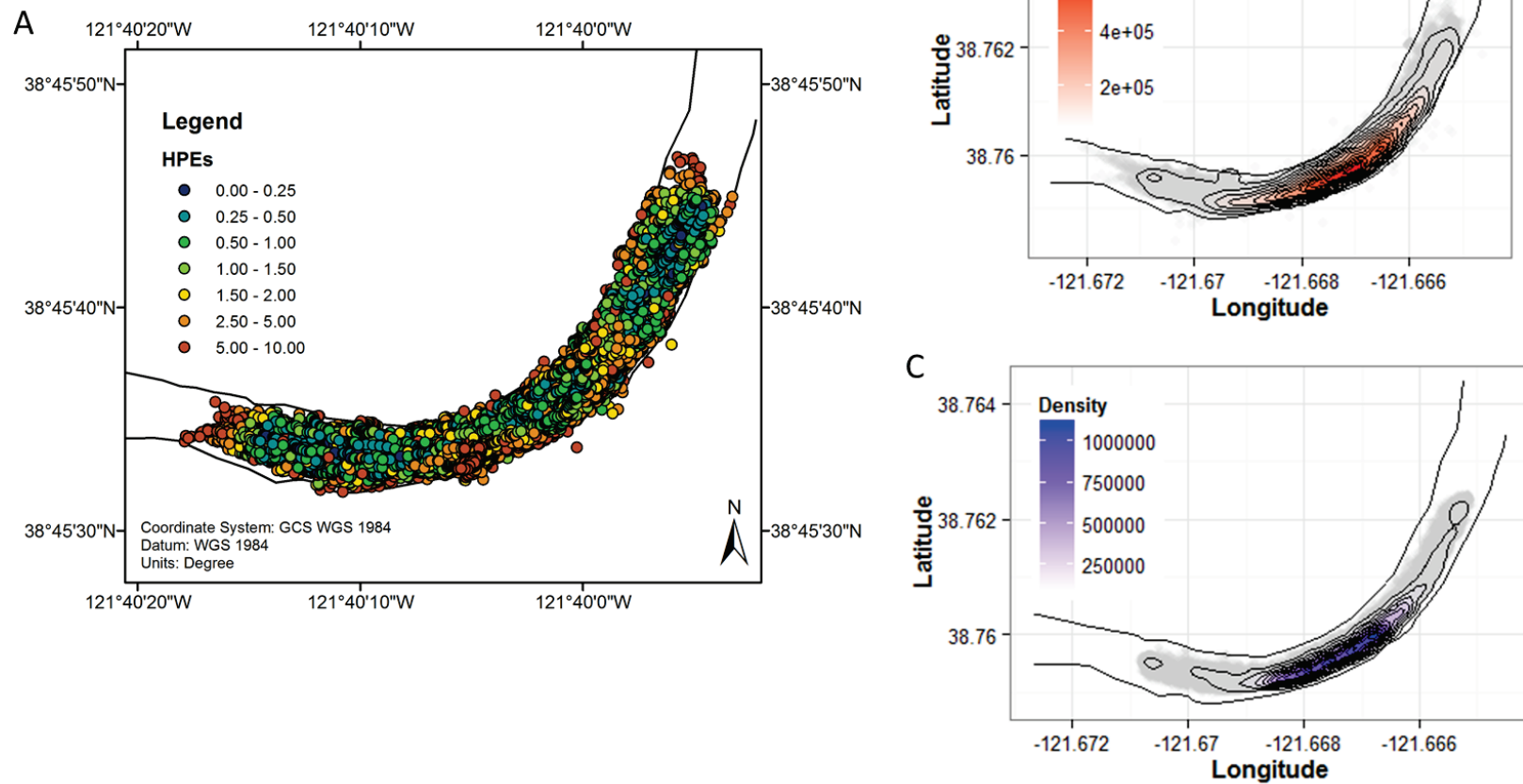
The 2D positioning array performed very well, with frequent detections and low error. A total of 168,234 positions were recorded during the study from 28 Jan 2015 to 6 Feb 2015, comprising positions for 490 of the 499 fish released. The remaining nine fish are presumed to have died within the 8 rkm between the release site and the array. The median number of positions per fish was 233, equating to approximately one position every 3 m if the positions were evenly spaced (Figure 8). Estimates of system precision made by Vemco's post-processing team used positions calculated for the sync and reference tags and were on the order of 2 m.

Figure 8. The number of positions recorded per fish, with a median of 233 positions.



There was low spatial variability in array performance within the study area. During post-processing, Vemco calculated a metric of quality for each position referred to as the Synthesized Position Error Sensitivity, and termed HPEs due to historical naming convention. This error estimate is described as a “relative, unitless estimate of error sensitivity” (Smith 2013) and should not be interpreted in terms of distance. Overall, the calculated values of HPEs were low, indicating that positional errors were low. There was also no specific region of poor detection or high error within the array; thus, when positions were filtered using a set threshold of HPEs, there was no substantial difference in the distribution of points removed versus those retained (Figure 9).

Figure 9. Map showing error associated with recorded positions. (A) Color indicates associated HPEs' value (an estimate of positional error). Positions shown with HPEs<10; fewer than 1% of total positions are omitted. (B) Density of positions and associated contour lines for all positions with an estimated error (HPEs) > 0.5 and (C) all positions with an estimated error <0.5. Darker colors indicate greater density of positions.



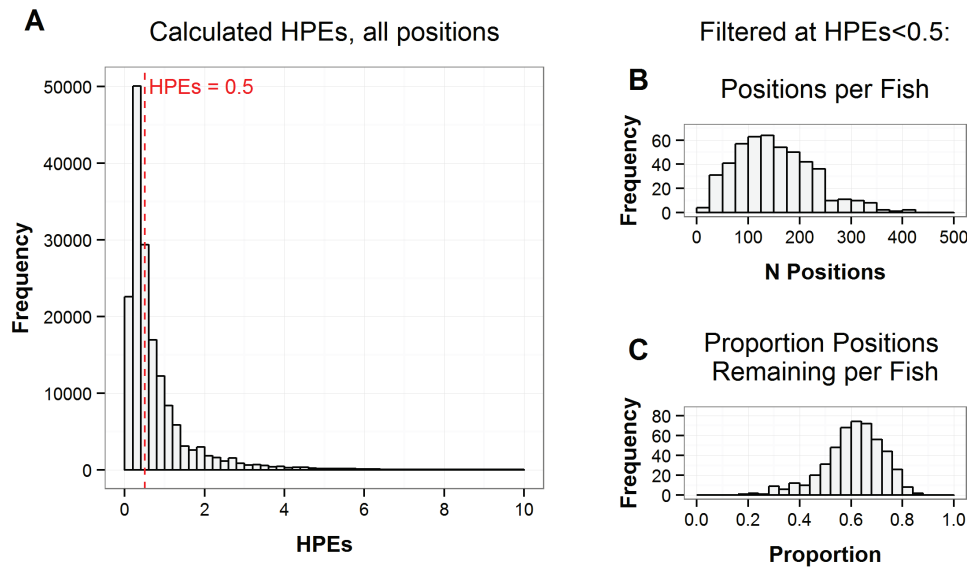
3.2 Data filtering

3.2.1 Primary filtering

Data manipulation and analysis was primarily conducted with the software program R (R Core Team 2015), and spatial analyses were done within the geographic coordinate system WGS84, with an azimuthal equidistant projected coordinate system for X, Y projected coordinates of fish positions. Primary data filtering was based upon the estimated positional uncertainty, HPEs, associated with each position. This value is calculated during post-processing at Vemco, as described previously. All positions with HPE values greater than 0.5 were removed from analysis (Figure 10). This threshold was selected based upon a detailed assessment of positional precision for both stationary reference tags and mobile fish tags, conducted by Vemco during post processing. Of the thresholds assessed, the research team determined that this choice provided the best tradeoff between data quality and quantity. Using an HPE threshold of 0.5 retained 52.8% of the original positions (88,752), with a median of 144 detections per fish (Figure 10, inset). This averages to approximately one position every 5 m, if the positions were evenly spaced, indicating that there is still a high density of positions remaining per fish after the primary filtering.

A visual assessment of tracks before and after preliminary filtering suggests no change in the fundamental properties of the tracks. Additionally, assessment of the spatial distribution of positions removed versus those retained suggests there was not a substantial bias in HPE values; thus, the filtering process should not lead to erroneous conclusions about space use by the study individuals (Figure 9). Based on the assessment by Vemco, the median estimated error of remaining animal positions was 1.21 m, while 95% of the positions had estimated errors <4.17 m, and 90% had estimated errors < 2.84 m. These estimates are biased high due to the estimation method, which used the variance of the distances between all pairs of points detected within 2 sec of one another. Therefore, a portion of this error is attributable to true movement of fish, while the remainder is due to error in the positioning itself. Much of this true error likely results from imprecision in the assumed fish depth (1.5m) or from multipath effects of the acoustic transmission as it travels between the tag and the receiver. There are additional environmental factors that can result in error (e.g., water temperature), but the authors expect these errors to be smaller than others discussed here.

Figure 10. Filtering level and resulting data set. (A) Distribution of estimated HPE values (estimation of positional error) for all positions recorded by the array, with vertical red line indicating chosen filtering level of HPEs < 0.5; (B) positions per fish after filtering at selected level; and (C) proportion of positions remaining per fish after filtering at selected level.



3.2.2 Secondary filtering

In addition to filtering the dataset by the HPEs values, the research team used several secondary filtration methods to remove problematic detections. The team used three criteria to identify tags which had likely been consumed by predatory fish: tags that remained in the array for extensive time periods (2 fish @ 317 and 2891 min); tags that moved into the array but never proceeded downstream out of the array (2 fish); and groups of fish that moved simultaneously through the entire 2D array and remained together through subsequent presence-absence detection stations downstream (2 sets of 2 fish each). It was possible that these sets of fish were eaten by the same predator and the tags were transported in its gut as it moved downstream. The team also removed detections for an individual that escaped into the river during the transition from transport containers into in-river net pens; the individual thus transited the array a day earlier than any other tagged fish. Overall, this filtering step removed nine additional individual fish from the analysis.

The secondary filtering process also identified single positions that resulted in biologically unreasonable rates of movement, defined as any ground speed between detections that was $>5 \text{ m sec}^{-1}$. This threshold is the

99.5th percentile of all measured movement speeds in the dataset. Manual assessment of points which exceeded this threshold also indicated that the high speeds generally corresponded to a single position that was unaligned with the track, suggesting positioning error rather than burst swimming behavior as the cause of the increased ground speed. Additionally, Castro-Santos et al. (2013) showed a maximum sprinting capacity of similar-sized salmonids to those in the present study of around 25 body lengths per second. For the present study's fish (mean FL = 133 mm), this equates to approximately 3.3 m sec⁻¹. This max sprint speed, combined with water velocities measured through the study reach, support a threshold of 5 m sec⁻¹ as a maximum biologically reasonable swim speed. An automated process was implemented, which scanned all tracks and removed these points. This removed an additional 322 positions from the dataset (0.4%). After the secondary filtration process, a mean of 150 positions remained per fish.

3.2.3 Track discretization

Juvenile Chinook migrate from their natal streams along continuous movement paths. Two-dimensional acoustic tracking arbitrarily divides these continuous movement paths into discrete linear segments between positions recorded irregularly in space and time. For some of the statistical analyses applied to the dataset, the tracks need to be rediscritized to create uniform time or distance intervals between positions (Turchin 1998). To illustrate the importance of this step, consider how the frequency at which positions are recorded along a continuous path will impact the magnitude of calculated turning angles. Thus, to control for the discrete nature of our positions yet still allow for comparisons between tracks, the team can rediscritize the positions to be at uniform distances. The simplest method to achieve rediscrization is to recreate a continuous path using linear interpolation between existing points, then subdivide this path at equal distance- or time-increments (Dray et al. 2009), depending upon the goals of the subsequent analysis.

The team used the R package 'adehabitatHR' (Calenge 2006) to conduct these rediscrizations of individual tracks. Fish that had long gaps between detections (>150 m) were removed from the analysis because of the large uncertainty in interpolating between widely spaced positions. This removed 38 of 481 individuals (7.9%). It is unclear why there were large gaps in the detection histories of this handful of fish, but there were higher proportions of these individuals in later release groups, suggesting there

may have been an influence of temporal variation in array performance. Fish with fewer than 10 positions after rediscrretization were also removed.

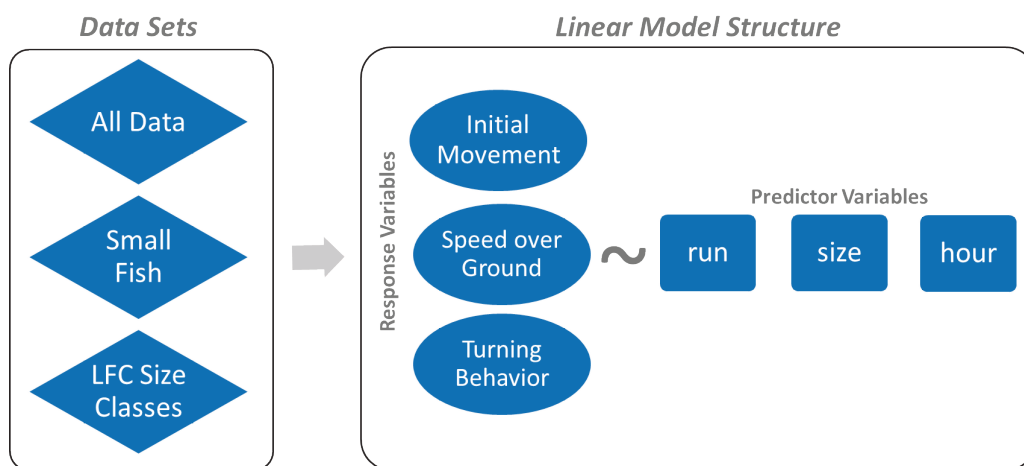
To spatially rediscrretize the track for analysis of turning angles, the team set the interval between positions to 21 m because this was the minimum distance possible due to computational limitations within the software package. The number of positions remaining was 12,632, or 17.5% of the previously filtered positions, leaving 439 individual fish with an average of 28.7 positions per fish. To temporally rediscrretize the track for analysis of speed and space use, the team set the interval between positions to 20 sec. The team chose this threshold because it reduced the dataset to approximately 1 of every 3 detections, and seemed to be an appropriate trade-off between over-interpolation across sparse positions and loss of data. Also, it provided similar resolution to the temporally rediscrretized dataset. The number of positions remaining at this threshold was 20,335, or 28.2% of the previously filtered positions, leaving 442 individual fish with an average of 46.0 positions per fish. While the lag at which data are no longer autocorrelated in ground speed or position is much longer than 21 m or 20 sec, this is not a concern for the majority of the following analyses because positions from individual tracks are not assumed to be independent. When this is an important statistical assumption, the data are resampled to remove autocorrelation. The temporal rediscrretization process also included staggering the starting point of the track to a random position within the first 100 m of the array. This was done to reduce the discrretization bias in the calculation of subsequent utilization distributions.

3.3 Movement analyses

To assess differences in behavior between the late-fall-run and winter-run Chinook, the team considered three primary movement patterns. This included the initial movement rates from release to arrival at the array, as well as speed over ground and turning behavior within the array. These later two combine to determine the total transit time through the array; to avoid redundancy, the team did not analyze transit time itself. For these three movement metrics (initial movement, speed over ground, and turning behavior) the team used linear regression to quantify the effect of run, while simultaneously considering effects of size and hour of release (Figure 11). Discharge was not considered in the models because it was very consistent across all releases. The team applied similar model structures to three different datasets: (1) the full dataset; (2) a reduced dataset with only comparable sized fish (98 - 125 mm FL); and (3) a reduced dataset with

only late-fall-run fish of two size classes (98 - 125 mm FL and 160 - 180 mm FL). The subsets of data were included to provide a second approach to controlling for differences in size or run, because these two metrics are not independent (Figure 11). Unless otherwise stated, an alpha of <0.05 was considered significant.

Figure 11. Schematic of nested analysis. Three datasets were each used to build three linear models. Each linear model used the same predictor variable to predict one of three response variables.



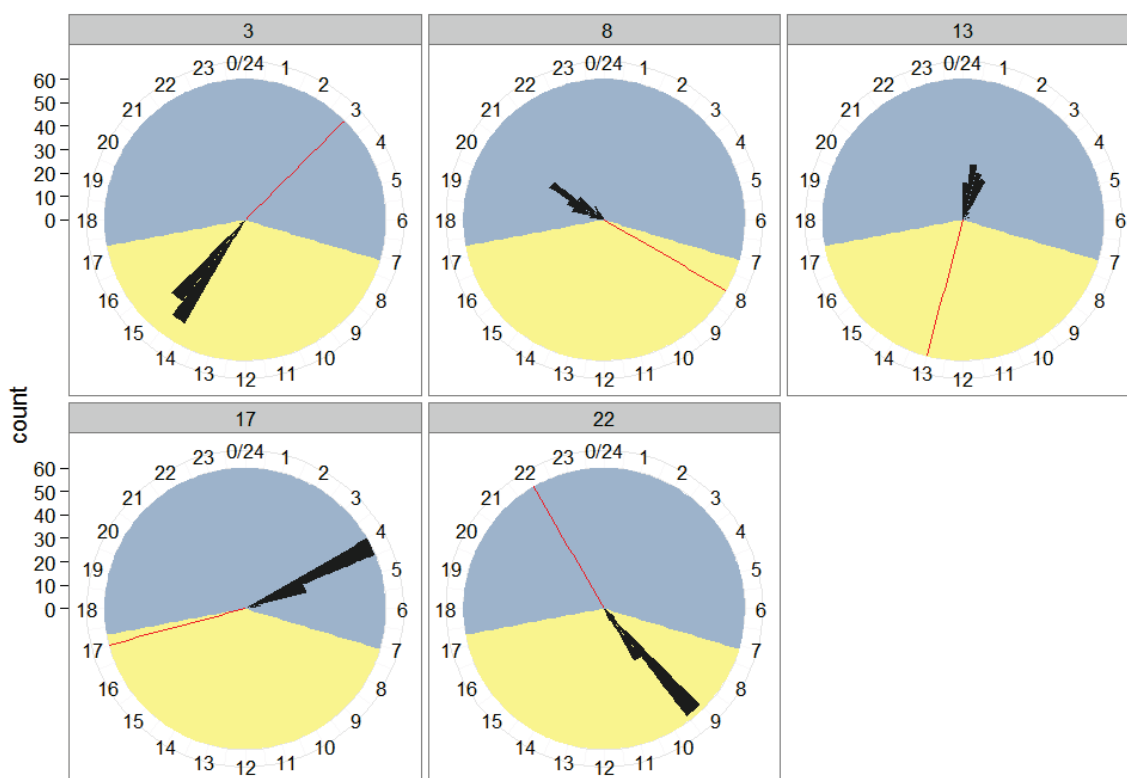
3.3.1 Initial movement

The filtered dataset was used to calculate the time of arrival at the array for each fish. Arrival times can indicate preferences for travel during specific diels, or can indicate variation in travel rate by time of day. To determine whether the fish released at the same hour but on different days could be analyzed as a single group, linear regression was used to assess differences in the time elapsed between release and the mean arrival time for each release group ($N_{LFC}=25$, $N_{WRC}=25$) and for each release hour ($N_{LFC}=50$, $N_{WRC}=50$). The model indicated significant differences in delay time by release group, with post-hoc contrasts indicating the only difference between paired hourly releases was at the 8:00 hour. This difference was driven by two outlying data points that had extremely long delays of 25.9 and 26.9 hr. When these outliers were removed, there were no significant differences between releases that occurred at the same hour, so the outliers were removed and each pair of releases were combined for further analysis.

Rose diagrams, histograms of circular data, show the arrival time by hour of release and illustrate a tight clustering of arrival times approximately

11-12 hr after the release time for all release groups, regardless of time of day (Figure 12). A Wilcoxon paired-sample test for skewness of circular data did not reject the null hypothesis that the data were symmetrical around the median ($p = 1$ for all groupings; Zar 1999).

Figure 12. Each panel shows circular histograms of arrival times for fish released at each hour, including fish from both runs. The red line indicates time of release. Overall, the mean travel time was approximately 11.7 hr.



To assess the influence of run on the time between release and arrival at the array, the team used a linear model with run, size, and release hour as predictor variables. The results from this model indicated that run is a significant indicator of delay time (Table 2), as was suggested by the raw data (Figure 13). There was not a significant effect of the interaction between run and fish size, and generalized variance inflation factors (GVIF; Fox and Monette 1992) for the model parameters were all less than 4. Therefore, the team doesn't believe the correlation between run and fish size has a strong impact on the model estimates or confidence intervals. However, the team also constructed linear models from subsets of data to test for the effects of run while isolating the influence of run or size. The model built

from data for fish of comparable size (98 - 125 mm FL) also indicated that run was a significant effect, further indicating it is not a spurious correlation between run and size that leads to this result.

This conclusion is further supported by the model built from the subset of data including only LFC of two size classes (98 - 125 mm FL and 160 - 180 mm FL). This model, as well as that built from the full data set, indicated that fish size was not a significant predictor. While the model built from the subset of data for small fish indicated that size was a significant predictor, this was over a much-reduced range of sizes and the effect was small. Additionally, it appears as though one outlier (LFC of 98 mm FL and delay time of 19.1 hr) exerts a strong influence on the results (Cook's distance > 0.5, Cook 1977). When it is removed, none of the three models indicate fork length as a significant effect.

Finally, for all three linear models, there were significant differences among release hours as predictors of the time between release and arrival at the array (Table 2, Figure 14). Post hoc comparisons between levels of release hour, using Tukey contrasts, indicated that those fish released during the day at 08:00 and 13:00 had significantly longer delays than those released during the night ($p < .001$), but the effect size was less than one hour (Figure 14).

Table 2. Parameter estimation for models predicting the initial movement between release and arrival at the array. Models of similar structure were built for the full dataset and two subsets of data. Parameter estimates are presented, as well as confidence intervals and p-values of the estimate, along with the adjusted R² value, indicating model goodness-of-fit. Significance of each level of release hour indicates difference from the overall mean. All significant p-values are shown in bold.

All Data (n=472) - adj. R ² = .319			Small fish only (n=56) - adj. R ² = .260		
	Estimate [95% Conf. Int.]	p-value		Estimate [95% Conf. Int.]	p-value
(Intercept)	12.74 [11.96, 13.51]	<0.001	(Intercept)	20.43 [14.10, 26.76]	<0.001
Run (WRC)	-0.90 [-1.17, -0.64]	<0.001	Run (WRC)	-2.04 [-3.05, -1.03]	<0.001
Fork Length (mm)	0.00 [-0.01, 0.00]	0.109	Fork Length (mm)	-0.07 [-0.12, -0.01]	0.015
Release Hour			Release Hour		
3:00	-0.37 [-0.5, -0.24]	<0.001	3:00	-0.66 [-1.25, -0.06]	0.031
8:00	0.50 [0.36, 0.63]	<0.001	8:00	0.25 [-0.37, 0.88]	0.422
13:00	0.42 [0.29, 0.56]	<0.001	13:00	0.69 [-0.01, 1.40]	0.053
17:00	-0.22 [-0.35, -0.09]	0.001	17:00	0.15 [-0.59, 0.90]	0.683
22:00	-0.33 [-0.46, -0.19]	<0.001	22:00	-0.44 [-1.07, 0.19]	0.166

LFC size classes (n=72) - adj. R ² = .177		
	Estimate [95% Conf. Int.]	p-value
(Intercept)	12.12 [11.78, 12.46]	<0.001
Size Class (sm) (Small)	0.40 [-0.14, 0.95]	0.142
Release Hour		
3:00	-0.80 [-1.27, -0.32]	0.001
8:00	0.58 [0.03, 1.13]	0.039
13:00	0.64 [0.07, 1.20]	0.028
17:00	-0.01 [-0.54, 0.51]	0.959
22:00	-0.40 [-0.95, 0.14]	0.146

Figure 13. Boxplots showing differences in initial movement by run or size class. Each panel displays trends from a different subset of data: (a) all data, (b) small fish only, and (c) LFC size classes. Two data points for LFC are not shown in panel a (25.9 hr and 27.0 hr).

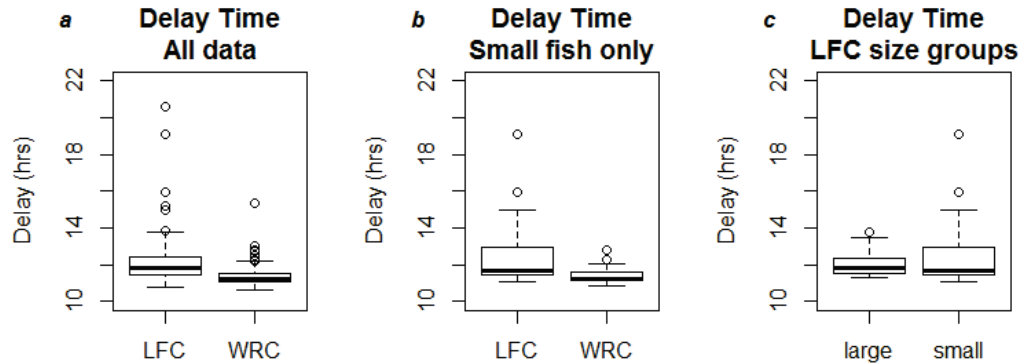
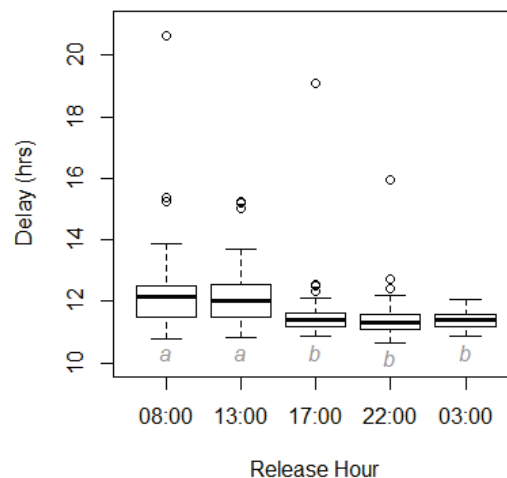


Figure 14. Boxplots indicating variation in initial movement between release and arrival time at the array for each unique release hour (N=100: NLFC = 50, NWRC=50). Boxes show 25th to 75th percentiles, while whiskers extend 1.5*IQR. Bold bar indicates the median. Two outliers were removed from the 8:00 release hour (Delay = 25.9 and 26.9 hr). Letters below the boxes indicate significance groupings from the Tukey HSD post-hoc test.



3.3.2 Speed over ground

Differences in behavior between LFC and WRC may be indicated by differences in migration speeds through the array. This can be quantified either through an individual's total transit time, or by estimating the path and calculating approximate ground speed within the array. The team considers the later metric here as it provides richer information about behavior. Some individuals may move slowly but directly while others may move rapidly with greater sinuosity, yet each could demonstrate similar total

transit times. To reduce the influence of inconsistent positioning efficiency within the 2D array on calculated path-lengths, the filtered tracks of individual fish were rediscritized to create positions separated by equal time intervals of 20 sec (see Section 3.2.3). While this level of rediscritization retains autocorrelation in speed over ground at sequential steps, the analysis aggregates all segments from an individual into a single track, thus providing one metric per fish and eliminating statistical problems commonly associated with autocorrelation (Legendre 1993). Using this rediscritized dataset, the length of each fish's path through the array was calculated and combined with passage time to provide an estimate of average speed over ground.

A linear model was used to assess the influence of run on ground speed, while also accounting for size and hour of release. Hour of release was used instead of release event because paired release events were not significantly different at the $\alpha = 0.01$ level. There was not a significant effect of the interaction between run and fish size, and generalized variance inflation factors (GVIF) for the model parameters were all less than 3.5. Therefore, the team doesn't believe the correlation between run and fish size has a strong impact on the model estimates or confidence intervals. The model showed there was no detectable effect of run on ground speed through the array, nor was there an effect of size (Table 3). This statistical result supports trends seen in the raw data (Figure 15). Post hoc comparisons between levels of release hour, using the full dataset with Tukey contrasts, indicated that fish released at 8:00 and 13:00 were significantly different from one another, and each moved significantly slower than those released at 3:00, 17:00, or 22:00 ($p < 0.001$, Figure 16).

Table 3. Parameter estimation for models predicting speed over ground through the positioning array. Models of similar structure were built for the full dataset and two subsets of data. Parameter estimates are presented, as well as confidence intervals and p-values of the estimate, along with the adjusted R² value indicating model goodness-of-fit. Significance of each level of release hour indicates difference from the overall mean. All significant p-values are shown in bold.

All Data (n=442) - adj. R2 = .301			Small fish only (n=48) - adj. R2 = .234		
	Estimate [95% Conf. Int.]	p-value		Estimate [95% Conf. Int.]	p-value
(Intercept)	0.67 [0.56, 0.79]	<0.001	(Intercept)	0.33 [-0.59, 1.26]	0.470
Run (WRC)	0.00 [-0.04, 0.04]	0.946	Run (WRC)	0.05 [-0.08, 0.17]	0.446
Fork Length	0.00 [0.00, 0.00]	0.640	Fork Length)	0.00 [-0.01, 0.01]	0.516
Release Hour			Release Hour		
3:00	0.06 [0.04, 0.08]	<0.001	3:00	0.08 [0.01, 0.14]	0.020
8:00	-0.11 [-0.13, -0.09]	<0.001	8:00	-0.12 [-0.19, -0.06]	<0.001
13:00	-0.06 [-0.08, -0.04]	<0.001	13:00	-0.03 [-0.10, 0.04]	0.361
17:00	0.07 [0.05, 0.09]	<0.001	17:00	0.07 [-0.01, 0.16]	0.091
22:00	0.04 [0.02, 0.06]	<0.001	22:00	0.01 [-0.06, 0.08]	0.817

LFC size classes (n=63) - adj. R2 = .201		
	Estimate [95% Conf. Int.]	p-value
(Intercept)	0.63 [0.59, 0.67]	<0.001
Size Class (sm)	0.01 [-0.06, 0.07]	0.804
Release Hour		
3:00	0.04 [-0.02, 0.09]	0.187
8:00	-0.13 [-0.19, -0.07]	<0.001
13:00	0.00 [-0.06, 0.07]	0.973
17:00	0.07 [0.00, 0.13]	0.042
22:00	0.02 [-0.04, 0.08]	0.441

Figure 15. Boxplots showing differences in speed over ground by run or size class. Each panel displays data from a different subset of data: a) all data, b) small fish only, and c) LFC size classes.

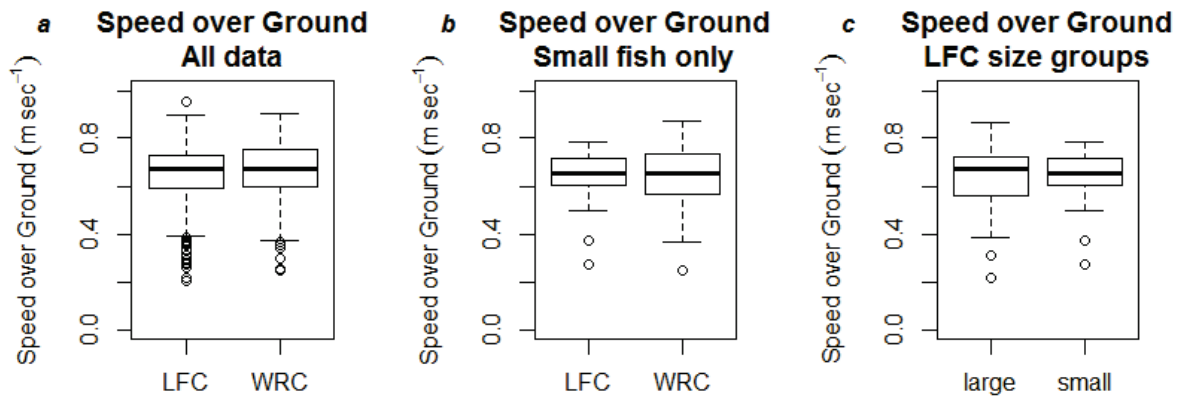
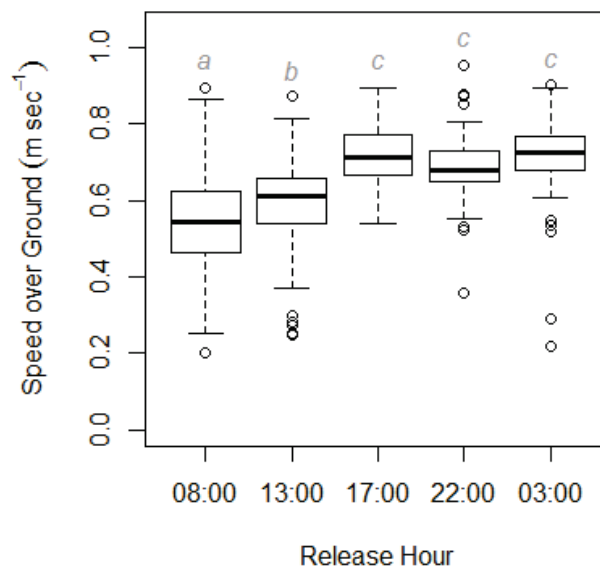


Figure 16. Boxplots indicating variation in ground speed (calculated over the entire reach) for each unique release hour (N=100: N_{LFC} = 50, N_{WRC}=50). Boxes show 25th to 75th percentiles, while whiskers extend 1.5*IQR. Bold bar indicates the median. Letters above boxes indicate significance groupings from the Tukey HSD post-hoc test.



3.3.3 Turning behavior

Low sinuosity of a fish track may indicate a greater propensity to migrate. To estimate sinuosity, the team uses the mean magnitude of the turn angles as an approximate measurement, where positive values are turns to

the left and negative values are turns to the right. To compare turn angles across continuous tracks, the team must use positions separated by constant distances, as the distance of each step will influence the measured turn angle between steps and therefore must be set at a constant distance. Thus, to assess differences in turn angle by run and size, the team used re-discretized tracks with 21 m steps between positions (see section 3.2.2 for additional details). To provide additional context for the mean turn angles from each fish track, the team calculated the mean turn angle for the centerline of the river, also discretized to 21 m. This mean turn angle was 2.9 (SD=11.8) degrees.

To compare the magnitude of turn angles between runs, the team analyzed the mean angle for each track, using an inverse transformation of the data (equivalent to step length traveled per 1 degree turned). The linear models also accounted for fish size and hour of release. Hour of release was used instead of release event because paired release events were not significantly different at the $\alpha = 0.01$ level. Generalized variance inflation factors (GVIF) for the model parameters were all less than 4. Turn angles did not vary by run for either model when run was considered (Table 4), as illustrated by the raw data (Figure 17). In the model built from the full dataset, there was a significant yet small effect of size (0.021, SD= 0.008), with smaller fish displaying larger mean turn angles ($p=0.005$, Table 4). However, fish size showed an effect in the opposite direction, and of a larger magnitude, within the model built from the subset of data including only LFC of two size classes (-1.71, SD=1.46, $p=0.022$; Figure 17). This suggests that fish size may impact turn angle, but is not conclusive. Adding further complexity, when the models were tested with an interaction term between run and size, this interaction term has a small effect size and was not statistically significant.

Finally, the model constructed for the complete dataset indicated that mean turn angles within the array were significantly different between release times (Figure 18). A Tukey HSD test indicated that the releases at 08:00 had a slightly larger mean turn angle than those released at 03:00 or at 17:00 ($p = 0.041$, $p = 0.045$); however, this was a very small effect. It was not considered significant in either model built from data subsets, likely because the power to detect differences was reduced with smaller sample sizes.

Table 4. Parameter estimation for models predicting turn angles within the positioning array. Models of similar structure were built for the full dataset and two subsets of data. Parameter estimates are presented, as well as confidence intervals and p-values of the estimate, along with the adjusted R² value indicating model goodness-of-fit. All significant p-values are shown in bold.

<i>All Data (n=439) - adj. R2 = .903</i>			<i>Small fish only (n=54 - adj. R2 = .956</i>		
	Estimate [95% Conf. Int.]	p-value		Estimate [95% Conf. Int.]	p-value
(Intercept)	3.86 [1.64, 6.08]	0.001	(Intercept)	1.32 [-7.68, 10.32]	0.769
Run (WRC)	0.62 [-0.14, 1.38]	0.108	Run (WRC)	1.10 [-0.22, 2.42]	0.099
Fork Length	0.02 [0.01, 0.04]	0.005	Fork Length	0.04 [-0.04, 0.12]	0.294
Release Hour			Release Hour		
3:00	-0.33 [-0.72, 0.06]	0.095	3:00	-0.11 [-0.83, 0.62]	0.763
8:00	0.51 [0.10, 0.91]	0.150	8:00	0.01 [-0.68, 0.71]	0.966
13:00	0.14 [-0.25, 0.54]	0.473	13:00	-0.39 [-1.14, 0.36]	0.297
17:00	-0.33 [-0.70, 0.05]	0.085	17:00	0.19 [-0.72, 1.09]	0.680
22:00	0.01 [-0.37, 0.39]	0.955	22:00	0.30 [-0.47, 1.07]	0.439

<i>LFC size classes (n=69) - adj. R2 = .825</i>		
	Estimate [95% Conf. Int.]	p-value
(Intercept)	7.78 [6.86, 8.70]	<0.001
Size Class (sm)	-1.71 [-3.17, -0.25]	0.022
Release Hour		
3:00	-0.53 [-1.84, 0.78]	0.419
8:00	0.67 [-0.78, 2.11]	0.359
13:00	0.90 [-0.65, 2.44]	0.25
17:00	-0.92 [-2.30, 0.46]	0.187
22:00	-0.11 [-1.56, 1.34]	0.881

Figure 17. Boxplots of the mean turn angles of each fish, grouped by run or size. Positive turn angles indicate turns towards the left-bank. Each panel displays data from a different subset of data: a) all data, b) small fish only, and c) LFC size classes. The red dotted line indicates the mean turn angle of the river calculated along the river center line. One data point for LFC is not shown in panel a (12.2°).

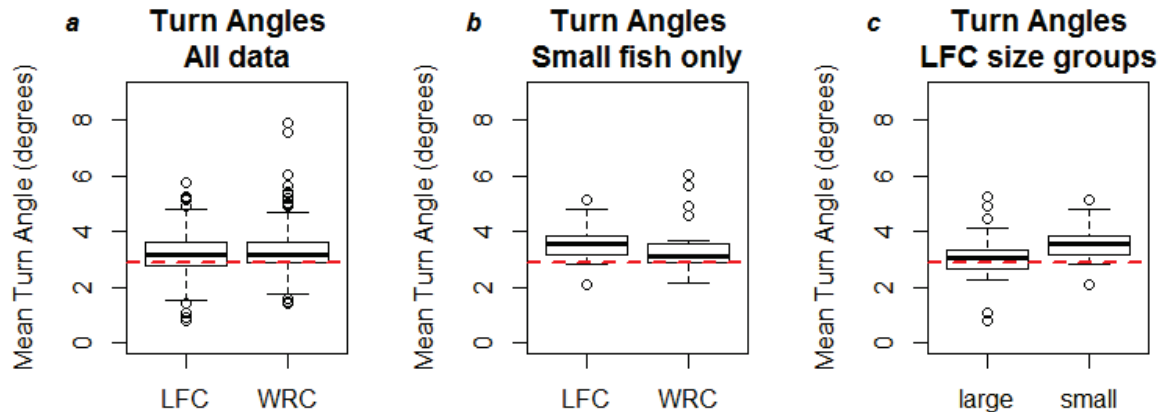
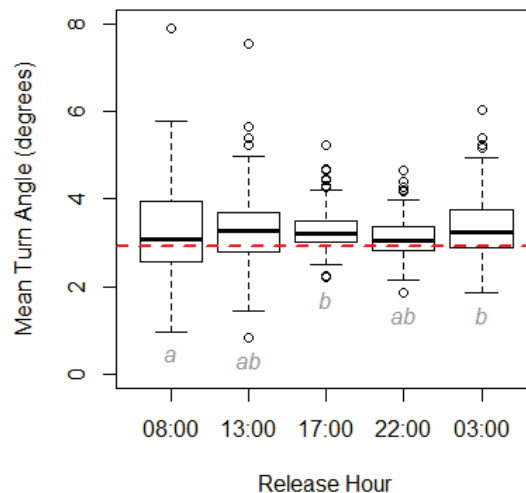


Figure 18. Boxplots indicating variation in turning angles along a track for each unique release hour ($N=100$: $N_{LFC} = 50$, $N_{WRC} = 50$). Positive turn angles indicate turns towards the left-bank. The red dotted line indicates the mean turn angle of the river calculated along the river center line. Boxes show 25th to 75th percentiles, while whiskers extend $1.5 \times IQR$. Bold bar indicates the median. Letters below boxes indicate significance groupings from the Tukey HSD post-hoc test.



3.4 Space use analysis

3.4.1 Cross-channel distribution

To better understand how juvenile salmon move in the vicinity of the Fremont Weir, the team examined their distribution within the channel. The positional data were collapsed to one dimension by projecting each

fish's 2D position onto its distance to the center of the channel (D2C), creating an empirical distribution of fish locations across the channel. Evidence for any bank preference was then assessed by looking for evidence that D2C data are skewed.

The D2C data across the entire sampling area are moderately skewed towards the outside bend of the river (Figure 19a; skew = -0.68, $t = -5.05$, $p < 0.05$, $N = 482$). These data were pooled across runs because there was little evidence of any difference in their distributions (Figure 19b). The data do show temporal correlations that vary widely across individuals, with no clear patterns emerging as a function of time of day or run type. Because this violates assumptions of independence required to calculate the team's test statistics, the team eliminated any correlations within individual tracks by performing a modified bootstrap. In each iteration, the team randomly sampled one position from each track and reported the mean skew and associated t -value from those 481 data points (repeated with replacement for 1,000 iterations). The team noted that directional variograms did show a strong anisotropic trend in spatial correlations across all relocation data, but this is not surprising given the proximity of the relocations and the nature of the advective environment. However, spatial and temporal correlations were deemed more problematic within individual tracks than across them.

The team also considered variation in the cross-channel distribution of fish as they move downstream. Each fish's relocation points were coded by step, beginning with their first relocation position upstream of the bend and ending with their last downstream position. At each step, the team then calculated the average D2C value across all fish, as well as the average position downstream. Confidence in each mean value is then weighted by the number of relocations (w). Mean D2C values shifted towards the outer bank before the bend and this bias persisted for the remainder of the recorded positions in this area (Figure 20), although there was an increase in variability farther downstream and as the number of supportive observations decreases.

Figure 19. Fish distribution patterns in space. (a) shows the full distribution of D2C values, and their relative position with respect to the center of the channel (N = 481 fish), while (b) shows the D2C densities across the channel plotted as a function of run. Light blue bars represent distribution of WRC, red bars represent distribution of LFC, and dark blue areas show overlap between runs.

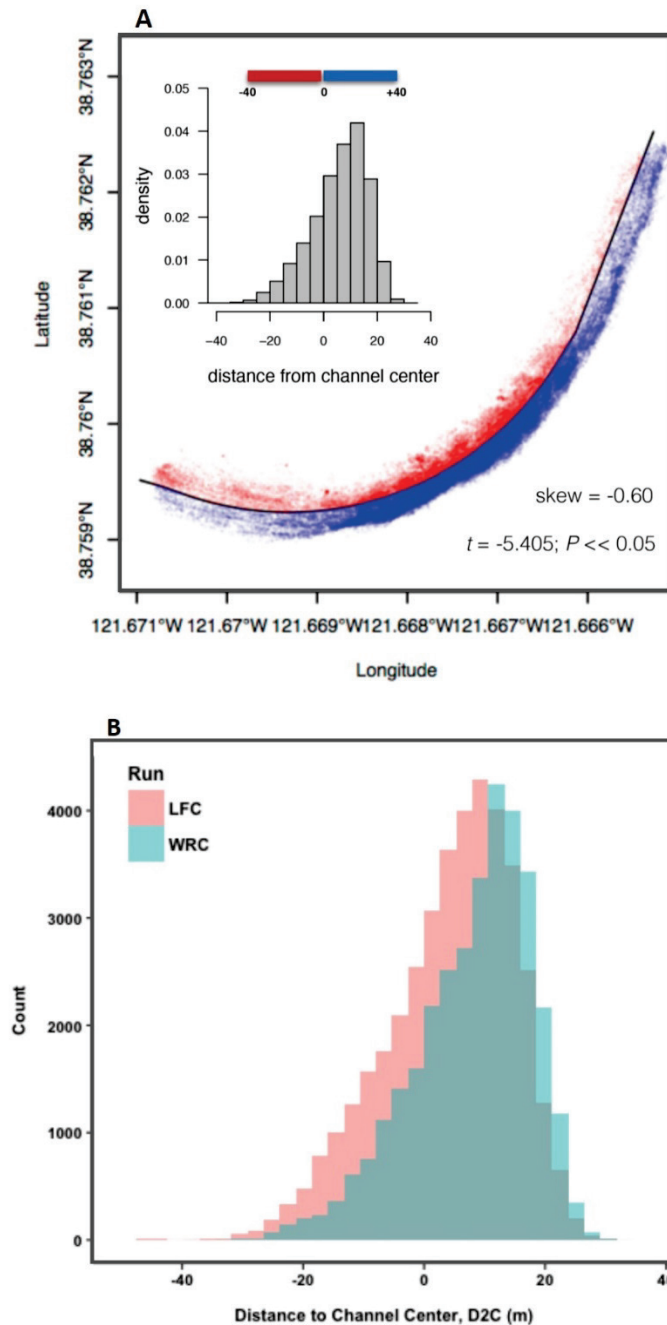
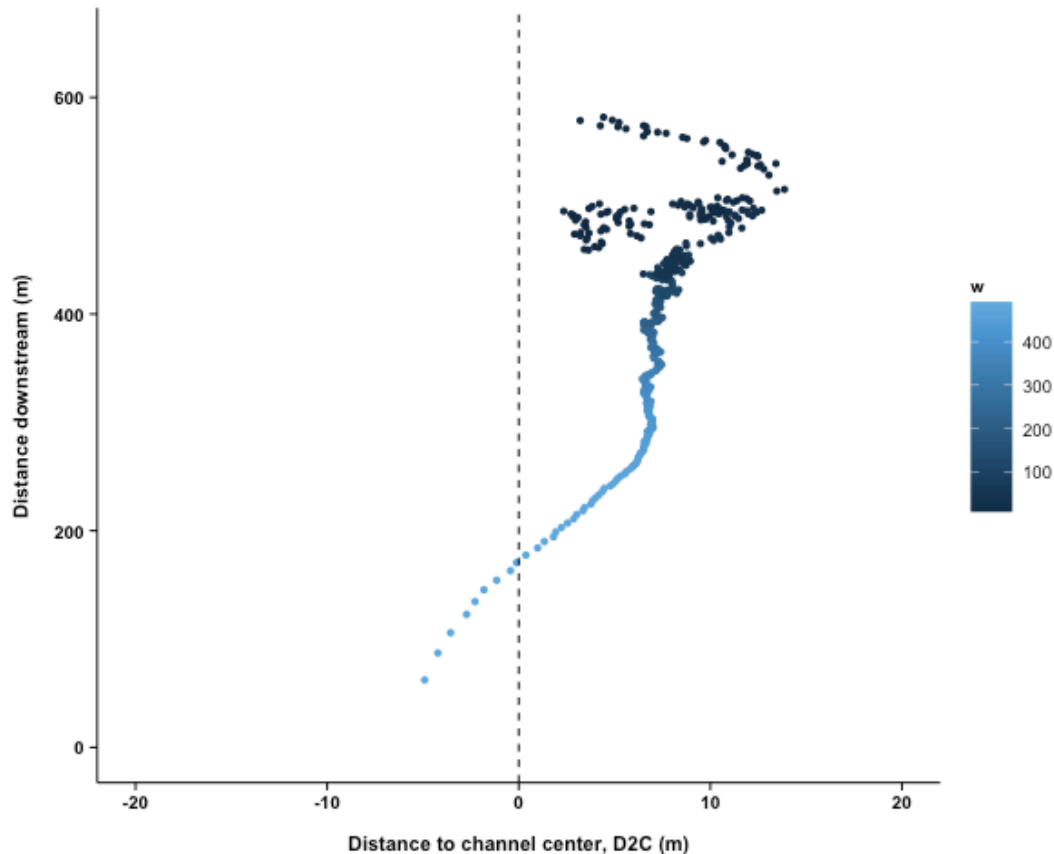


Figure 20. Trends in mean D2C values as a function of the downstream distance. Bias towards the outer bank is indicated by positive D2C values, while negative values indicate a bias towards the inner bank. Confidence in each mean value is weighted by the number of relocations (w), and indicated by the color gradient. Notice that as the number of supportive observations decreases (smaller w , darker color) the trend becomes more variable, although still biased towards the outer bank.



3.4.2 Kernel Utilization Distributions

To assess the degree of spatial overlap between the two runs of Chinook within the positioning array, the team created kernel utilization distributions (UDs) from the temporally rediscritized detections of each run. The rediscritized tracks were used to reduce bias that may result from fish that were detected more frequently. This approach uses known locations to create a probability density map of space use, and can output polygons of contours along that two-dimensional distribution. The team used the function provided in the *adehabitatHR* package within R (Calenge 2006), with a least squares cross-validation approach for identifying a smoothing parameter. In addition to creating UD from positions of all fish in each run, the team created parallel UD for the two subsets of data discussed above (small fish from both runs, and LFC in two size classes).

By examining the UD's produced from all positions of each run, the team again saw that both runs were more likely to be along the outside edge of the bend as they migrated through the array. Generally, the area used by LFC was larger than that used by WRC (Table 5). This seems to be an effect of run, not size, because large and small LFC showed similar space use. There was also a subtle trend for the WRC distributions to be more condensed along the outer bend (Figure 21).

Despite these minor differences, at all contours examined, there was substantial overlap between the runs. In the contour of the 50th percentile, 81% of the area of the WRC distribution was overlapped by the same percentile contour of LFC. In the contour of the 90th percentile, 93% of the area of the WRC distribution was overlapped by the same percentile contour of LFC (Table 5; Figure 22). The same trend of high overlap between runs at the 90th percentile contours was also demonstrated for the subset of similarly sized fish from each run.

Table 5. The area of utilization distributions at the 50th and 90th percentile contours for the three subsets of the data. Also shown are the percent of area overlapped by the same contour calculated for the other run or size class.

All Fish			
Run	Contour	Area (hectares)	% Overlapped by alternate run
LFC	50	9.9	58%
WRC	50	7.1	81%
LFC	90	27.8	82%
WRC	90	24.8	93%
Small Fish			
Run	Contour	Area (hectares)	% Overlapped by alternate run
LFC	50	11.2	57%
WRC	50	9.7	66%
LFC	90	31.0	77%
WRC	90	25.1	95%

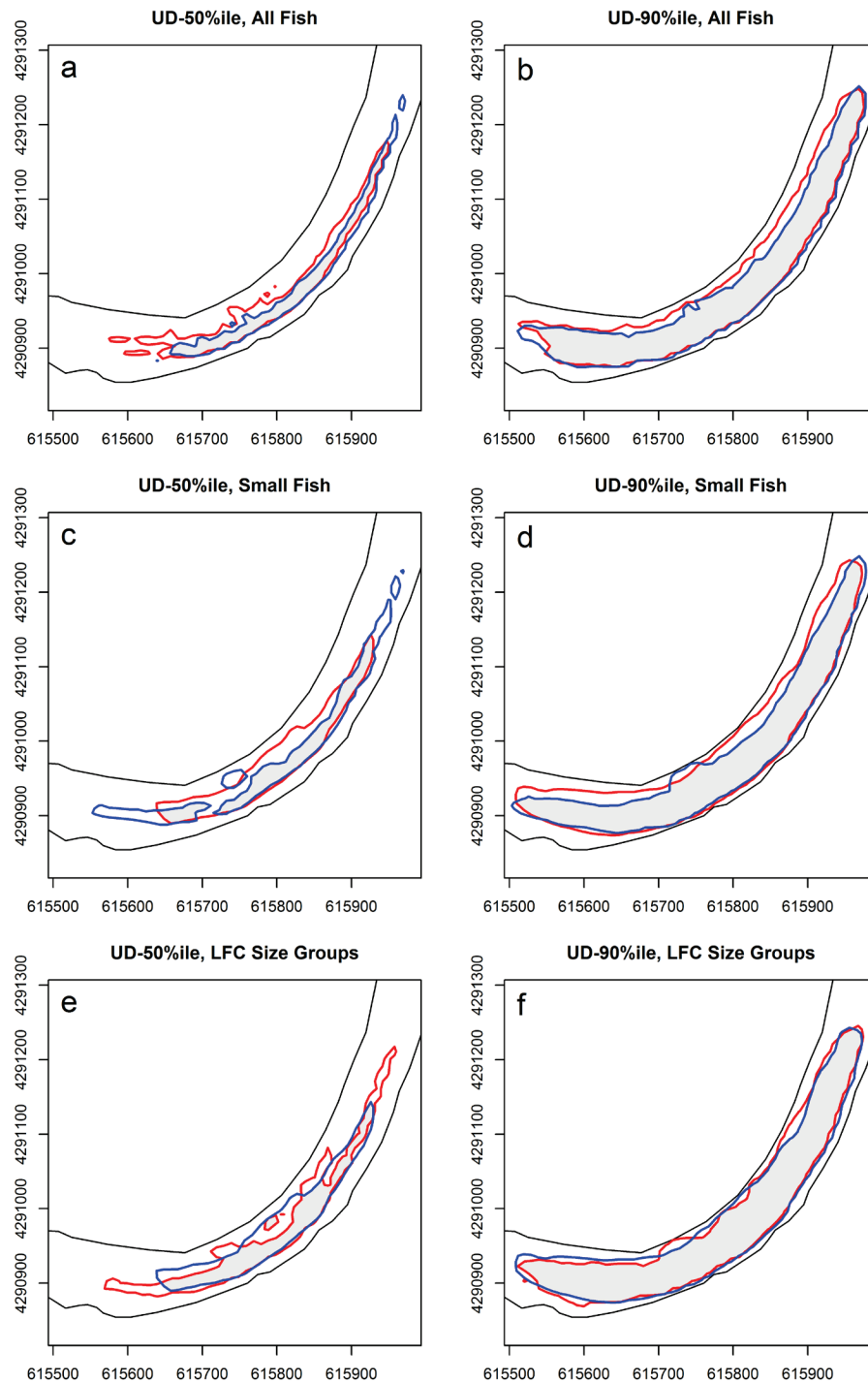
LFC size class			
Group	Contour	Area (hectares)	% Overlapped by alternate size class
Large	50	11.3	67%
Small	50	11.2	67%
Large	90	29.9	93%
Small	90	31.0	89%

Figure 21. Full utilization distributions calculated for all fish from each run. Hotter colors indicate greater probability of use. Contour lines are shown for perspective, and approximate river banks are shown.



Figure 22. Selected utilization distribution (UD) contours (50th and 90th percentiles).

In panels a-d, UD for LFC are represented by red lines and UD for WRC are represented by blue lines. Panels a-b show results for all individuals of each run, while panels c-d show results for 'small fish,' defined as fish between 98 - 125 mm FL. In panels e-f, UD for large LFC (160 - 180 mm FL) are represented by red lines and UD for small LFC (98 - 125 mm FL) are represented by blue lines. The area of overlap is shaded grey.



4 Discussion

4.1 Array performance and study design

This project's study design and execution resulted in very good quality information on fish movement at the location of the Fremont Weir. The Vemco equipment and positioning array design were efficient in the detection of surgically implanted tags. The surgical and release procedures were effective in limiting immediate handling-associated mortality of juvenile Chinook. The release location did not result in high mortality before study fish arrived at the positioning array, which was a driving factor in selecting the study release site. As a result, there is a possibility of moving the release location further upstream for future work. During the planning phase of this study, there were concerns that fish were being released too close to the study site, so fish may not have sufficient time to acclimate to river conditions. Therefore, fish may not behave like wild or "run of the river" fish. However, the release location was not changed due to concerns about mortality. Results in 2015 would support a release location that is further upstream.

Furthermore, the release schedule resulted in fish arriving at the positioning array across a variety of diels, allowing for the detection of any behavioral changes that may have been associated with time of day.

Unfortunately, the river flows remained consistent throughout the entire study period, reducing the flow variability at which behavioral data was collected.

4.2 Behavior patterns

Overall, the behavior within the positioning array was dominated by direct, downstream movement, and generally rapid speed over ground. This is similar to two-dimensional observations for juvenile late-fall-run Chinook salmon migrating through a reach approximately 2.5 rkm upstream (Sandstrom et al. 2013). This behavior suggests the study fish had a strong propensity to migrate, and is in agreement with our understanding that juveniles in the size range from 100 - 200 mm FL have begun the process of smoltification (Muir et al. 1994, Giorgi 1997). While there was no effect of size on speed over ground, the team did see a subtle effect of size on turning angle. Together, these two metrics determine total travel time, synonymous with exposure time. Thus, because smaller LFC had slightly larger

turn angles but similar speed over ground, the team expects these smaller fish may also have greater exposure time as they migrate. Both the increased turning behavior and the increased exposure time could result in size selective mortality beyond what is expected, due to the effects of predator gape limitation (Anderson et al. 2005).

One purpose of this study was to determine whether there were distinct behavioral differences between winter-run and late-fall-run Chinook salmon. Overall, there seemed to be very little difference between the two runs. Behavior was quantified with three primary metrics, including transit time from release to positioning array, speed over ground through the array, and mean magnitude of turn angles within the array. The only metric that was significantly related to run identity was the transit time from release to the positioning array, with WRC moving more quickly than LFC. The result remained significant even when size and release time were accounted for. This may suggest that the two runs differ in their response to novel situations, such as release into the river. However, overall, the team saw very little behavioral differentiation.

Differences between WRC and LFC juveniles were also assessed through space use. In considering both the cross-channel distribution of individuals and kernel utilization distributions, the fish showed a non-random and non-uniform distribution. Both runs had a tendency to be closer to the outside bend than the inside bend. These data also indicate a tendency for winter-run fish to be slightly farther toward the outer bend than late-fall-run (Figure 21). While there is a noticeable size difference between the runs, the similarity in space use of large and small LFC individuals suggests that the differential space use between runs is driven by run identity — not variation in size, at least within the size range tested here. Differences in swimming depth may be a potential explanation, as there is depth-related variability in hydrodynamic conditions. However, for this study, individual fish positions were not recorded in three dimensions; thus, the team cannot directly test this hypothesis.

There was little evidence of consistent differentiation in movement patterns between sizes of juvenile Chinook. It is also important to note that the individuals considered in this study were within a size range frequently classified as smolts or pre-smolts (95 - 188 mm FL), and thus may not have shown the full range of variation expected across all sizes of naturally outmigrating juvenile salmon. When compared directly, small LFC showed

a greater increase in mean turn angle than large LFC. There are several hypotheses for the mechanism behind this slight difference. It could correspond with previous work that showed smaller fish at earlier stages of ontogeny show less propensity to migrate (Giorgi 1997), and consequently may be expected to display less directed movement paths. However, given the limited size range of fish in this study and the limited evidence in the scientific literature about the relationship between size and behavioral state, the team does not expect that this small variation in turn angle is due to ontogenetic habitat preferences. Alternatively, the greater mean turn angle toward the left bank shown by the smaller LFC could correspond with a change in the river hydrodynamics as it flows out of a left bend. At the upstream end of the receiver array fish concentrated towards the river-right bank as it entered the turn, while at the downstream end of the array, these advective forces were relaxed. As the hydrodynamic forcing of fish towards the right was reduced, individuals may have moved from channel right to channel center or left, resulting in mean turn angles that were larger than the mean turn angle of the river itself (Figure 17). It is feasible that fish of unequal size classes could be differentially affected by these hydrodynamics, with smaller fish having slower burst swim speeds experiencing a higher degree of susceptibility to advection by hydraulic forces. Finally, size-based differences in mean turn angles could also occur if size impacted an individual's active behavioral response to flow fields. Smaller fish have shorter lateral lines, thus impacting their ability to resolve flow fields and perhaps altering the outcomes of a decision process based on perceived hydrodynamics. It will be valuable in the future to collect movement data on a wider size range of juvenile salmonids to help resolve this question.

The most consistently influential variable across all models was the hour of release. Those fish released during the day (08:00 or 13:00) took more time to move between the release site and the positioning array. Interestingly, daylight hours also composed a large portion of the transit period for fish released at 03:00, but these fish did not show longer transit times. This suggests there may be a relationship between light intensity and behavior at the time of release. Previous work in the Sacramento River has shown differing responses of juvenile salmon to time of day (Chapman et al. 2013).

The slower movement of fish released during the day persisted through the positioning array even though these fish reached the array at night. Slower

ground speeds were not observed in the groups released at 17:00, which also arrived at the array during night. Those fish released at 8:00 showed slightly larger turn angles than two of the groups of fish released at night (03:00 and 17:00). In addition, eight of the nine sets of fish detected schooling through the array in groups of two or more were released during day time releases. These observations suggest that, in addition to immediate effects, the time of release may have lingering effects on the behavior of the juvenile salmon.

4.3 Surrogacy potential

The detectable differences between winter-run Chinook and late-fall-run Chinook during their migration past Fremont Weir were very minor. They included slightly different responses to the novel situation of release from net-pens into the river, and slight differences in space use. Given this information on behavioral response and space use, it seems reasonable to use hatchery late-fall-run juveniles as surrogates for studies on the behavior of hatchery winter-run juveniles within the mainstem of the Sacramento River at the size classes tested for the study. This is an important observation because there are many studies of late-fall-run Chinook movement in the Sacramento Basin that could be consulted.

4.4 Future work

This study was conducted in a single year under unusually low stable discharges. It may be beneficial to have similar data under additional flows in order to gain insight into how these fish respond to different or more complex hydrologic conditions. The Fremont Weir notch will operate over a wide range of potential discharges and additional data at those discharges has potential to assist with design. This recommendation is tempered by the observation that late-fall-run Chinook may be a suitable surrogate for winter-run Chinook over the size ranges tested in this study.

Additional hydraulic complexity could be added as part of a study design as well. A floating boom could be installed to assess fish response. In addition, future notch operations may benefit from the knowledge of fish guidance potential near the Fremont Weir. Specifically, fish guidance may improve notch efficiency by entraining more fish with less water.

Perhaps the biggest uncertainty is related to the lack of very small fish (30 to 70 mm) in the data set. Natural spawning WRC often pass the

Fremont Weir in this size range. Due to our understanding of juvenile salmon ontogeny, there is reason to believe that smaller fish may behave differently than the large fish tracked in this study. However, the team is limited with regard to the size of fish that can be observed using the current technology due to concerns about the potential for large tag burdens to alter behavior (Adams 1998). It would be beneficial to develop a plan for future assessment of fish typically classified as fry in order to gain a more holistic understanding of the behavior of all migratory juvenile Chinook within the mainstem of the Sacramento River. This additional work would also profit from the inclusion of naturally spawned individuals, as hatchery salmonids have been shown to display different behaviors than naturally reared fish (Alvarez et al. 2003, Swain and Riddell 2011).

Information from this study will be used with hydraulic modeling (Lai 2016) to provide quantitative information regarding ELAM modeling of juvenile fish entrainment at Fremont Weir. Modeled Fremont Weir “notches” will vary in base elevation, dimensions, and location and will be evaluated at multiple hydrostatic conditions to learn about sensitivity to these factors and potential success and risks of these modeled scenarios. In 2016, a second phase of interagency ecohydraulic investigations is continuing at Fremont Weir and these data sets should be useful for further improving our fish behavior and hydraulic modeling tools. These additional data sets will enhance these tools for interagency teams to quantify and evaluate adult and juvenile fish passage designs.

4.5 Conclusion

Juvenile Chinook moved quickly and in a highly directional manner through the study reach. They displayed a non-uniform distribution within the channel, with a tendency to use space along the outer bend more frequently than the inner bend. This successful study provided evidence for little difference between the behavior of late-fall-run and winter-run Chinook salmon juveniles at the Fremont Weir. Thus, further plans to aggregate information from multiple runs to inform parameterization of fish behavioral models for use in evaluating juvenile entrainment at potential Fremont Weir “notch” alternatives is reasonable and will capture variation reflecting fish size and run timing. The ability to generalize from these data to all juvenile Chinook of the Sacramento River would be further enhanced by similar studies with additional hydrodynamic complexity, smaller fish, and wild fish, as the team has reason to believe each of these scenarios may result in subtle behavioral differences that should be reflected in expectations of migration behavior of juvenile salmonids in rivers.

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Appendix A: Tagging Winter-Run Chinook Salmon at the Livingston Stone National Fish Hatchery

Standard Operating Procedure (SOP)

Adapted from Liedtke et al. (2012), the 2011 procedures from Cramer Fish Sciences, the SOPs used for Department of Interior's south Delta telemetric studies, and the 2011 work instructions used for the U.S. Army Corps of Engineers telemetric studies

Purpose and scope:

This SOP provides the steps needed to tag hatchery winter-run Chinook salmon at the Livingston Stone National Fish Hatchery for the 2015 Fremont Weir Fish Behavior Study. Over the course of two days, 250 winter-run Chinook salmon will be tagged at the hatchery and held in circulars until the salmon are ready for release. At a minimum, the following staff will be required to implement this SOP:

- Two surgeons to tag salmon and to work on equipment setup;
- Two data recorders to help with entering data in a Microsoft Access database and to help prepare the acoustic tags; and
- Two fish runners to help with moving tagged Chinook salmon to the circulars and to help with preparing recovery buckets.

When applicable, this SOP identifies the tasks that are assigned to the data recorders and fish runners. Any tasks not assigned to these staff are directed to the surgeons. However, the surgeons can seek assistance from the data recorders and fish runners when appropriate.

Materials:

1. Hach HQ40d meter with dissolved oxygen/water temperature and pH probe
2. User manual available at <http://www.hach.com/asset-get.download.jsa?id=7648131637>
3. Thermometer for quick temperature checks

4. pH meter or litmus paper
5. Acoustic tags (V-4)
6. VEMCO acoustic tag activator with manual. Manual found at http://vemco.com/wp-content/uploads/2013/03/vta_manual.pdf.
7. VEMCO acoustic tag verification equipment (VR-100 with VH180 hydrophone) with manual. Manual found at http://vemco.com/wp-content/uploads/2013/02/vr100hw_manual.pdf.
8. Chlorhexidine solution (Nolvasan; 30 mL/L D-H₂O)
9. Distilled or de-ionized water (D-H₂O)
10. Tricaine methanesulfonate (MS-222; 100 g/L)
11. Sodium bicarbonate solution (buffer; 100 g/L)
12. Stress coat - stock concentration and 25% solution (250 mL/L D-H₂O)
13. Disinfectant solution (i.e., 70% ETOH)
14. PVP iodine (Argentyne)
15. 19 L black bucket(s) marked at 10 L and clearly labeled “Anesthesia”
16. 19 L buckets for post-surgical recovery of fish and for rejecting fish
17. Liedtke et al. (2012) does not recommend the use of white or black buckets. White buckets are not ideal for restricting light penetration, while black buckets are too dark and absorbs large amounts of solar radiation. However, the color of the bucket will not be an issue for this study since tagging occurs indoors.
18. Cooler for storing fish before tagging
19. Two large water containers marked at 38 L
20. Water pump, with extension cord and rubber tubing with in-line shut-off valve and terminal narrowing
21. Rubber tubing to return water from drain tray to maintenance anesthetic bath
22. Designated syringes (5 mL) for measuring anesthetic and stress coat
23. Oxygen delivery system (cylinder, regulator, airline, air diffusers) for recovery buckets
24. Fish nets (e.g., sanctuary nets, dip nets)
25. Nitrile gloves (in all sizes)
26. Scale measuring to the nearest 0.01g (weighing fish and tags)
27. Large sponge to weigh fish
28. Measuring board with ruler to the nearest mm
29. Surgical platform (cradle)
30. Trays for holding solutions used to disinfect surgical tools
31. Trays to rinse disinfected tools
32. Needle drivers (multiple sets)

33. Forceps (multiple sets)
34. Scalpel handle and blades (multiple sets)
35. Scissors (multiple sets)
36. Tissue collection supplies: scissors, blotter paper, labeled coin envelopes
37. Sutures: 19 mm 3/8 circle needle with 4/0 Mono-Dox (violet monofilament polydioxanone) suture material
38. Spray bottles for disinfectant solution
39. Timers and stopwatches
40. Sharps container
41. Datasheets, clipboards, and writing tools
42. Laptops
43. Carabiner tag labels to identify fish in recovery buckets
44. Clean rags for keeping tagging areas clean and dry
45. Tables

Pre-tagging activities:

- Prior to the tag implantation, the tagging coordinator will need to get in touch with the Livingston Stone National Fish Hatchery about the following items:
 - Notify hatchery staff on the pre-tag fish-holding period requirements. The pre-tag fish-holding period should be 18 to 36 hr.
 - Food should be withheld during the pre-tagging holding period.
 - Notify hatchery staff on the list of study personnel that will be at the hatchery. All study personnel must bring government issued identification, such as a California driver's license.
 - Coordinate with the hatchery staff on a list of materials that the hatchery should provide. In terms of using water quality meters, check with the hatchery on when the meters were calibrated.
- Disinfect all buckets and coolers with PVP iodine (e.g., Argentyne) before arriving to the hatchery. If this step is not done before arriving, then all equipment must be disinfected at the hatchery before use.

Equipment Setup:

- Datasheet Setup

- Start the electronic *Tagging Datasheet* in a Microsoft Access database for each tagging station. Each data recorder should have a separate database.
 - Prepare a hard copy *Daily Fish Reject Tally Datasheet* for each tagging station to account for fish that are handled, but are not used for the study.
 - Prepare a hard copy *Circular Chain of Custody*.
- Tag Activation
 - The data recorder should activate the transmitters the day before or the day that they are to be implanted using the VEMCO tag activator.
 - Afterwards, the data recorder will confirm the operational status with the VR-100 and a VH180 hydrophone.
 - Once this is done, sterilize the acoustic tag in a solution of Nolvasan for a few minutes. Following disinfection, thoroughly rinse transmitters in distilled or deionized water prior to implantation.
 - Record the tag serial ID, the tag code ID, the surgeon's name, and the data recorder's name in the electronic *Tagging Datasheet* after tag verification.
 - Calibrate the scale and weigh a tag to the nearest 0.01 g in the electronic *Tagging Datasheet*. This value will be used for every fish.
- Setting up Circulars
 - Check to make sure that the ten circulars for tagged Chinook salmon have water circulating through it. Afterwards, label each circular with a study circular ID with white duct tape and a Sharpie pen. For the study, there should be ten circulars.
 - In the end, each circular should have 25 fish and five circulars will be used for each day.
- Filling and Preparing Trays and Buckets
 - Fill disinfection trays for surgical instruments with Nolvasan.
 - Fill disinfection trays for surgical instruments with diluted Nolvasan.
 - Fill rinse trays with de-ionized or distilled water.
 - See Figure 1 for example of tray setup.
 - Clip on numerical tag labels to recovery buckets, which will serve as the bucket ID.
- Water Temperature Checks for Anesthesia Bucket, Surgical Bath, and Holding Cooler

- Water temperatures during all aspects of the tagging operations cannot exceed 2°C difference from the reference water source. The fish runners or surgeons will check all water sources periodically and record results in the *Tagging Datasheet* to ensure that water temperature levels are within criteria. For this study, the rectangular tank where source fish are held is the reference water source.
 - Anesthesia buckets, maintenance bath containers, and recovery buckets should not be filled until near the time that they are needed to avoid warming.
 - Anesthesia buckets and maintenance bath containers should be replaced regularly to prevent increasing water temperatures over time.
- Equipment Setup for Recovery/Reject Buckets
 - Set up the oxygen cylinder with a trigger.
 - The oxygen cylinder will be used for the recovery/reject buckets. Prior to use, conduct an experiment to see how long you should hold the trigger to target a DO saturation of 120 to 150% in the recovery bucket. Seven sec was used in past SOPs, but this may differ for this study.
 - This recovery bucket should be attached with an air stone and air pump.
 - With guidance from the hatchery, identify the tank that will be used for placing reject fish.

Fish Selection Criteria:

- For all experimental groups, handling protocols will be standardized to reduce potential bias (i.e., fish length, number of times handled, tagging procedures, transport methods, transport time, and release protocol).
- VEMCO V4 tags weigh about 0.41 g in the air. The estimated minimum length and weight of the Chinook salmon for surgical tagging should be >8.2 g (tag weight ≤ 5 % of the body weight), respectively. Fish should be targeted between 90–100 mm in FL.

Fish Tagging:

- *Equipment setup*
 - Prepare surgical table and equipment for use.

- The surgeon should wear clean gloves during all procedures that involve handling fish.
 - The surgical station will be cleaned and wiped down with a solution of disinfectant, and surgical instruments will be placed in a disinfectant bath (e.g., dilute Nolvasan, chlorhexidine solution) before fish handling and surgical procedures.
 - Surgical instruments will be transferred to a freshwater rinse bath before surgery and rinsed twice.
 - Rinse tray should be changed often to avoid accumulation of disinfectant in rinse water.
 - To minimize the chances for pathogen transfer between fish populations, all equipment used for capture, holding, anesthesia, surgery, recovery, and movement of fish during the project will be thoroughly cleaned and disinfected before use with a different fish population.
 - Soiled gloves should be changed immediately and after handling 10 fish.
- Set up measuring board and scale.
 - Put approximately 1–2 mL of diluted stress coat on the weighing sponge and the measuring board.
- For each tagging station, the fish runner must fill a 19 L bucket halfway with circular water. In addition, the bucket should be supplied with a small amount of undiluted stress coat and with oxygen using an oxygen cylinder. The bucket should also be fitted with an air stone/air pump before tagging.
 - The concentration of DO in the buckets should be between 120% and 150% saturation by holding the trigger for a few sec.
 - These 19 L buckets serve as both the recovery bucket and the reject bucket. The bucket becomes the recovery bucket if the fish was tagged and it becomes the reject bucket if the fish was handled, but not used for the study.
 - No fish should be euthanized since winter-run Chinook salmon are listed under the U.S. and California Endangered Species Act.
- Administration of Anesthetic
 - The effectiveness of MS-222 as an anesthetic varies with factors, such as temperature, fish density, and individual sensitivity. Adjustments of the anesthesia concentration should be based on the amount of time it takes for a fish to lose equilibrium. Any adjustments should be recorded in the *Tagging*

Datasheet with a separate treatment ID.

- Fill the anesthesia bucket with 2 L of circular water. As a suggestion for a starting concentration, add 7 mL (1 mL= 1 cc) of the MS-222 stock solution. This will yield an anesthetic concentration of 70 mg/L. Base the daily starting concentration on fish responses during the tagging operation from the previous days.
- All anesthetic solutions will be buffered between a pH of 7 and 8 using sodium bicarbonate dissolved in solution.
- Prepare the maintenance bath containers with water from the circular and with a water pump/tubing (see Figure 2 for setup). This is done by completing the following procedures:
 - Fill the container with 38 L of circular water.
 - Place the pump into water and ensure tubing is fit properly.
 - As a suggestion for a starting concentration, add 7.6 mL (1 mL= 1 cc) of MS-222 stock solution (100 g/L) and 7.6 mL of bicarbonate solution (100 g/L). This will yield an anesthetic concentration of 20 mg/L. Base the daily starting concentration on fish responses during the tagging operation from the previous days.
 - Water in all containers (anesthesia and maintenance) should be changed regularly to minimize dilution of anesthesia water and temperature changes. Moreover, this is done to ensure you do not run out of water during a procedure.
 - Add a small amount of diluted stress coat for each liter of water in the anesthesia and maintenance bath to protect fish from loss/damage to the slime layer.
- Anesthetizing the Fish
 - With help from the hatchery, identify the rectangular tank with fish that will be used for the study and place the fish into a cooler with an air pump and air stone.
 - Having fish in a cooler will help the surgeons gather fish for tagging and avoid a long commute from the tank to the tagging station.
 - Use a sanctuary net or dip net to remove one fish from the pre-tag holding cooler and place directly into an anesthesia bucket.
 - Remove the fish from the net by hand, taking care not to dilute the anesthesia bath with water from the net.
 - Note: The most significant source of stress that fish experience is usually from being netted.

Every effort should be made to minimize handling. Sanctuary nets should be used when feasible.

- Secure the lid as soon as the fish is in the bucket.
- Call out “fish in drugs” and start a timer to keep track of how long a fish has been in the anesthesia bucket. The data recorder will record the start time of when the fish was placed in the anesthesia bucket in the electronic *Tagging Datasheet* based on when the surgeon called out “fish in drugs.” Time is recorded in the 12-hour clock notation in the following format: hh:mm:ss am/pm.
- Remove the lid after about 1 minute to observe the fish for loss of equilibrium. Once the fish loses equilibrium, keep the fish in the water for an additional 30 to 60 sec. When you take the fish out of the anesthesia bucket, call out “fish out of drugs.” At that point, the data recorder will record the end time of when the fish was placed in the anesthesia bucket in the “Time out of Drugs” column in the electronic *Tagging Datasheet*. Time is recorded in the 12-hour clock notation in the following format: hh:mm:ss am/pm.
 - Relay any information to the data recorder. Time of sedation should normally be 2 to 4 min, with an average of about 3 min. If loss of equilibrium takes less than 1 minute or if a fish is in the anesthesia bucket for more than 5 min, then reject that fish. If after sedating a few fish and they are consistently losing equilibrium in more or less time than what is typical, then the anesthesia concentration may need to be adjusted. This should only be done after consultation with the field lead, and should be done in 0.5 mL increments. Concentration changes should be executed for all surgeons simultaneously and recorded on the *Tagging Datasheet*.
- Start a timer when a fish is removed from the anesthesia bucket to document the time the fish is out of the water. Once the fish is out of the anesthesia bucket, measure fish length, weight, and condition in the *Tagging Datasheet* using the steps described below:
 - Transfer the fish to the scale and weigh to the nearest 0.01 g.
 - Transfer the fish to the measuring board and measure fork length (FL) to the nearest mm.
 - Evaluate eye, scale, and fin condition and rate them as “good” (g), “fair” (f), or “poor” (p).

- If a fish is unacceptable for tagging, then place the fish in the reject bucket and inform the data recorder to update the *Daily Fish Reject Tally Datasheet* and to update the *Tagging Datasheet*.
 - In addition, inform the fish runner to transport the fish in the reject bucket to the reject tank. Fish should be transferred through water-to-water transfers.
 - Data must be vocally relayed to the data recorder and the data recorder should repeat the information back to the surgeon to avoid miscommunication.
 - Any fish that is dropped on the floor during this process must be rejected.
 - A fish dropped on the table during surgery may still be tagged.
 - If a fish is dropped on the floor after it is tagged, then remove the tag and reject the fish. Afterwards, the fish should go in the reject bucket and should be placed back into the reject tank by the fish runner.
 - The data recorder should document this information in the *Daily Fish Reject Tally Datasheet* and update the *Tagging Datasheet*.
- Implanting a Surgical Tag
 - Selected fish will be bathed in cool ($< 14^{\circ}\text{C}$), aerated water during surgery. Surgery will be performed in as sterile an environment as possible.
 - Fish will be placed ventral-side up on a surgery cradle made of Microcell foam with a size-specific mold to hold the fish in position.
 - See *Figure 3 to 5* at the end of this SOP for general reference of surgical procedures.
 - Water diffused with a maintenance anesthesia solution (20 mg/L) will be passed through the tubing using a pump and will continually flow into a reservoir in the mold where the fish's head will be submerged. This will gently flush the anesthetic solution over the gill membranes to ensure oxygen and anesthesia is carried to, and metabolic wastes are efficiently moved away from, the gills continuously throughout the procedure. Using the in-line valve, adjust the flow as needed, so that the gilling rate of the fish is steady.
 - Using a Sharp point 15° stab point (3.0 mm or 5.0) mm restricted blade depth scalpel, an approximate 5 mm incision will be made parallel to and 2 mm to the side of the ventral midline, and anterior to the pelvic girdle.
 - One scalpel blade can be used on 5 to 7 fish before it

- becomes dull. If the blade is pulling roughly or making jagged incisions, it needs to be changed.
- Use blunt-tipped forceps or hemostat to open the incision to ensure you did not damage any internal organs or cause excessive bleeding.
 - Do not implant the tag and reject that fish if you observe damage or think you damaged an organ. Excessive bleeding indicates likely organ damage. Therefore, it should be noted on the *Tagging Datasheet* if the surgery continues.
 - In order to avoid cutting into the pelvic girdle with the scalpel incision, consider making the incision from the tail towards the head. This will reduce the chance of tearing skin near the pelvic girdle. Even a small nick in the pelvic girdle will compromise swimming ability.
 - A disinfected transmitter will be inserted through the incision into the peritoneal cavity of the fish. Transmitters should only be handled by gloved hands or clean surgical instruments such as forceps after the disinfection step.
 - The tag will be positioned, so it is lying immediately under the incision.
 - If a battery side is evident on the tag, it should be inserted first with the battery oriented parallel to the incision. As the tag is placed into the peritoneal cavity, the battery should be pushed towards the tail and the transducer of the tag should be towards the head.
 - This positioning will provide a barrier between the suture needle and internal organs. Through time, the tag location will naturally move posterior in the fish.
 - The incision will be closed with one simple suture using the 3/8 circle needle with 4/0 Mono-Dox (violet monofilament polydioxanone) suture material.
 - Note: While suturing in and out, forceps should be used to separate the skin from muscle and organs to avoid suturing anything but the skin.
 - To make a stitch, lock the needle (at the end of the suture) in the hemostat so the needlepoint faces you. Enter the outside edge of the incision on the side farthest from you and exit through the other edge of the incision, pulling the suture perpendicular through the two edges. The needle should enter and exit the skin as close to the edge of the incision as possible without tearing the skin (~ 2 mm from edge of incision).
 - Pull the needle and suture through the skin to leave a tag end of about 2 to 3 cm of suture material, protruding from the needle entrance location. Afterwards, release the needle from the needle drivers.

- With your non-dominant hand, grasp the long end of the suture material (usually with thumb and forefinger) at or below the needle, and make two forward wraps (i.e., away from your body) around the tip of the needle driver, which should be held in your dominant hand.
- With the two wraps still around the needle driver, grasp the short tag end of the suture material with the needle driver. Tighten the stitch by pulling the wraps off the needle driver and pull both ends of the suture material, perpendicular to the incision.
- On the first knot, the dominant hand holding the needle driver should pull toward your body and the non-dominant hand should pull away from your body. Tighten the suture lightly, just so the edges of the incision meet, but do not overlap, pucker, or bulge the edges of the incision. The second knot is the same as the first, but in reverse order.
- On the second knot, grasp the long end of suture material with your non-dominant hand, make two reverse wraps (i.e., toward your body) around the end of the needle driver, grasp the short end of suture with the needle driver, and tighten the stitch. This time, the knot should be tightened by pulling your dominant hand (holding the needle drivers) away from you and your non-dominant hand toward you. The second knot can be slightly tighter than the first, again taking care not to overlap, pucker, or bulge the edges of the incision. This completes one knot.
- Cut the suture with the hemostat or scissors, leaving ends approximately 2 mm in length.
- If the incision is too long to close with one stitch, it is acceptable to add a second suture knot. Relay this information to the data recorder to document in the “Notes” section of the *Tagging Datasheet*. Furthermore, the surgeon will tell the data recorder if the incision, suturing, and tag placement was “good” (g), “fair” (f), or “poor” (p). Lastly, the surgeon should determine the level of bleeding (0, 1, 2, 3).
 - If the fish is in bad condition, then the fish should be rejected.
- Call out “surgery complete” and transfer the fish from the surgical platform to the appropriate recovery bucket for ten minutes. This should be done with minimal handling by moving the platform as close as possible to the bucket or using a liner material to lift the fish for transfer.
 - After the surgeon calls out “surgery complete,” the fish

runner should start the timer for ten minutes and the data recorder should record the actual time in the “Time out of Surgery” column of the *Tagging Datasheet*. Actual time should be recorded in the 12-hour clock notation (hh:mm:ss am/pm). In addition, there should be one fish per recovery bucket.

- When ten minutes is up, the fish runner will transport the fish to the circular (see next section).
- Each individual suture (one packet) can be used on five fish. Disinfect the suture material and the attached suture needle in the sanitizing solution used for instruments.
- Between surgeries, the surgeon should replace the tools that were just used into the disinfectant bath. Each surgeon will have at least 3 sets of surgical instruments to rotate through to ensure that tools get a thorough soaking in disinfectant for between uses (about 10-minute minimum contact time with disinfectant). Each surgery station will have one tray of Nolvasan, one tray of diluted Nolvasan, and one of distilled or de-ionized water.
 - Once disinfected in Nolvasan solution, rinse the tools thoroughly with distilled or de-ionized water and ensure that the scalpel blade and suture are ready to use on the next fish. Organic debris in the disinfectant bath reduces its effectiveness, so be sure to change the bath regularly. If necessary, replace the scalpel blade.

Placing Tagged Fish into the Circulars:

- After the fish has stayed in the recovery bucket for ten minutes, the fish runner should remove the lid and make sure the fish has “recovered.” This means that the fish has regained orientation and is maintaining upright swimming.
 - If the fish is no longer alive, then the fish runner should bring back the fish in the bucket to the surgeon. Afterwards, the surgeon will perform a necropsy and retrieve the tag (see *Performing a Necropsy of Tagged Chinook Salmon* SOP for procedures).
 - If the fish recovered, then the fish runner will move the bucket and clipboard over to the circular for holding the fish.
 - Once at the circular, the fish runner should release the fish into the tank. This is done by partially submerging the bottom of the recovery bucket into the circular and

gently tilting the bucket until the fish is released into the circular.

- When the fish is released, the fish runner will call out “recovery complete” with the bucket ID. Afterwards, the data recorder will record the time in the 12-hour clock notation in the “Recovery” column of the *Tagging Datasheet*.
- It is the responsibility of the fish runners to keep track of the number of fish that are placed into a circular. This should be done through tallies on the *Circular Chain of Custody*.
- When there are 25 fish in the circular, the tagging coordinator should record the total number of fish in the tank and document any mortality in the *Circular Chain of Custody*. Any dead salmon should be bagged in separate Ziploc bags and labeled with the date of bagging, the time of bagging using the 24-hour clock notation (hh:mm), and the circular in which the dead salmon was found. Afterwards, the surgeon will perform a necropsy and retrieve the tag (see *Performing a Necropsy of Tagged Chinook Salmon* SOP for procedures).
- After 25 fish are placed into a single circular, the fish runner should move on to the next circular using similar procedures. Five circulars should be filled per day with 25 fish.
- At the end of each day, the fish runner or data recorder should record the number of fish, the mean weight of fish (nearest 0.01 g) and the mean fork length of fish (nearest whole fish in mm) for each circular in the *Daily Feed Log*. This information is used to determine the rough amount of feed to place into each tank (see the *Daily Fish Care after Tagging at the Livingston Stone National Fish Hatchery* SOP).
 - In addition, the fish runner should measure the dissolved oxygen levels (mg/L and % saturation) and water temperature (°F) of one of the circulars at the end of the tagging session. Additional circulars should be checked if there are water quality concerns.
 - Record all parameters as measured with the Hach meter.

End of Session Activities:

- Validate the tag data and datasheet accuracy.
 - Working together, each tagger and assistant team will review the transmitter tubes/serial numbers against the *Tagging Datasheet* to verify that all of the transmitters provided for the session were implanted into study fish. The steps of the verification process could include reading the serial number on each tag tube and finding that the serial number on the datasheet to confirm that it was implanted.
- Review information on the *Tagging Datasheet* and complete any

missing information.

- Organize tagging solutions and surgical instruments to be ready for the next tagging session.
- Provide the Livingston Stone National Fish Hatchery with any datasheets that they need for the fish care process. This includes all of the datasheets needed for the fish care process and the necropsies that have to be performed in case there are morts.

End of Day Clean up:

- At the end of each tagging day, wipe down or spray all surfaces with 70% ETOH to disinfect.
- Move rejected fish back into the circulars based on the protocols by the hatchery. Dispose of any morts from the reject coolers in the dumpster.
- Use a toothbrush to remove all large organic debris from instruments, rinse them and dry them to prevent rust.
- Make surgical tagging solutions as needed to be ready for the next tagging session.
- Inventory chemical solutions and tagging supplies (blades and suture).
- Return any soiled rags to the office and have them washed.
- Rinse buckets with hose and place upside down to dry.
- Turn off the oxygen cylinder.

General Fish Handling Reminders:

- Anesthesia and freshwater containers and buckets should be filled just prior to tagging to avoid temperature changes and should be changed often. Check levels of carboys before each surgery to be certain that you will not run out of water during a surgery.
- **USE CAUTION and COMMUNICATION** when adding MS-222 and bicarbonate to any container to avoid adding two doses or no doses to the container.
- Keep a lid on any bucket or cooler that contains fish.
- Any fish dropped on the floor should be rejected. If a fish is dropped on the floor after it has been tagged, then remove the tag, and place it into another fish. The dropped fish then goes into the reject cooler for sutured fish.
- **CAREFULLY HANDLE BUCKETS.** Try not to bang them around, slam the handles, or otherwise handle in a rough manner as this can stress fish.

- **USE A SANCTUARY NET** to capture source fish and place them into an anesthesia bucket. A recommended approach is to use a non-sanctuary net in the container of source fish in order to capture the fish without them detecting the pressure wave in front of the sanctuary net. Once a fish is in the traditional net, place the sanctuary net immediately below the fish so that the handles of the two nets are aligned and can be handled together.

Figures

Figure A1. Example of setup for disinfecting and rinsing surgical equipment. The figure shows one container of Nolvasan, one container of diluted Nolvasan, and one container of distilled water.



Figure A2. Example of setup for maintenance bath (large container) with drain tray (smaller container) and surgical platform.

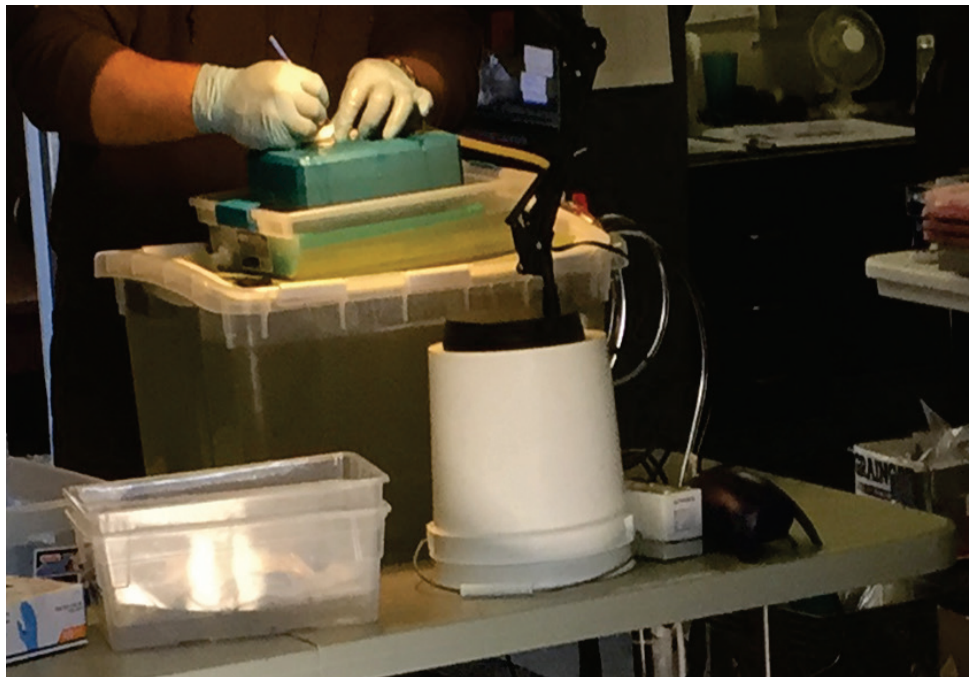


Figure A3. Lateral view of a juvenile salmonid, showing the location of internal organs. Courtesy of Liedtke et al. (2012).

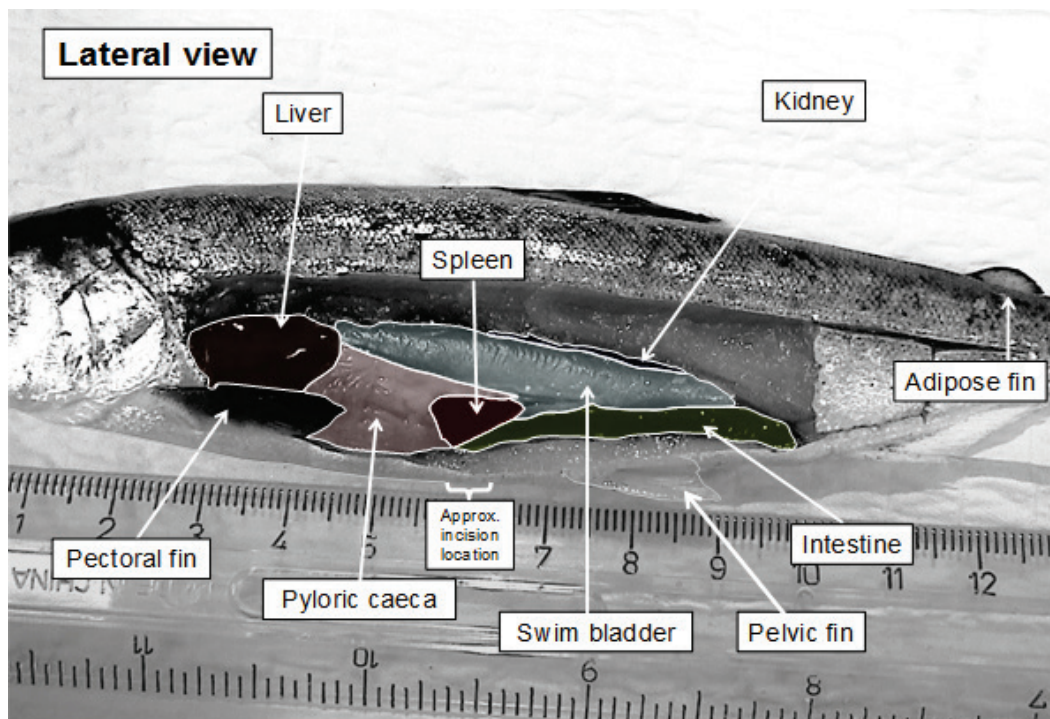


Figure A4. Proper surgical procedures help ensure rapid recovery and incision healing (note proper incision healing on photo right). Courtesy of Cramer Fish Sciences.

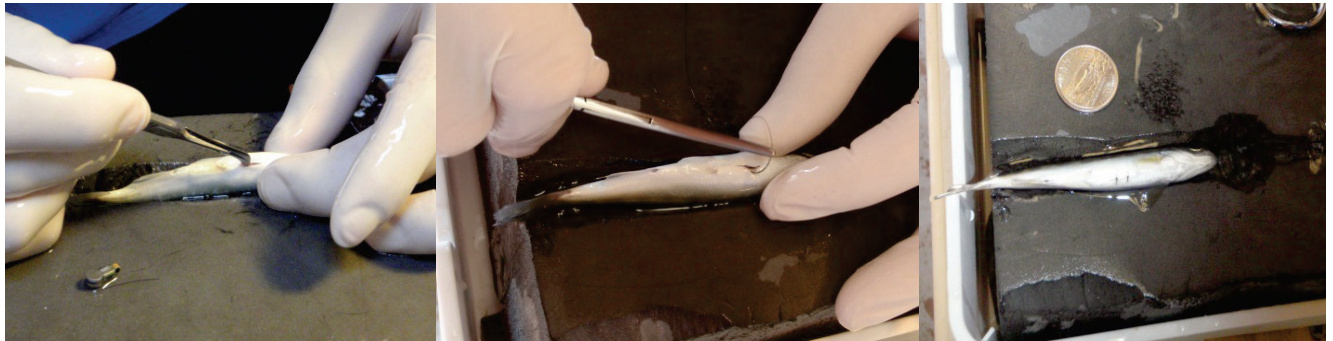
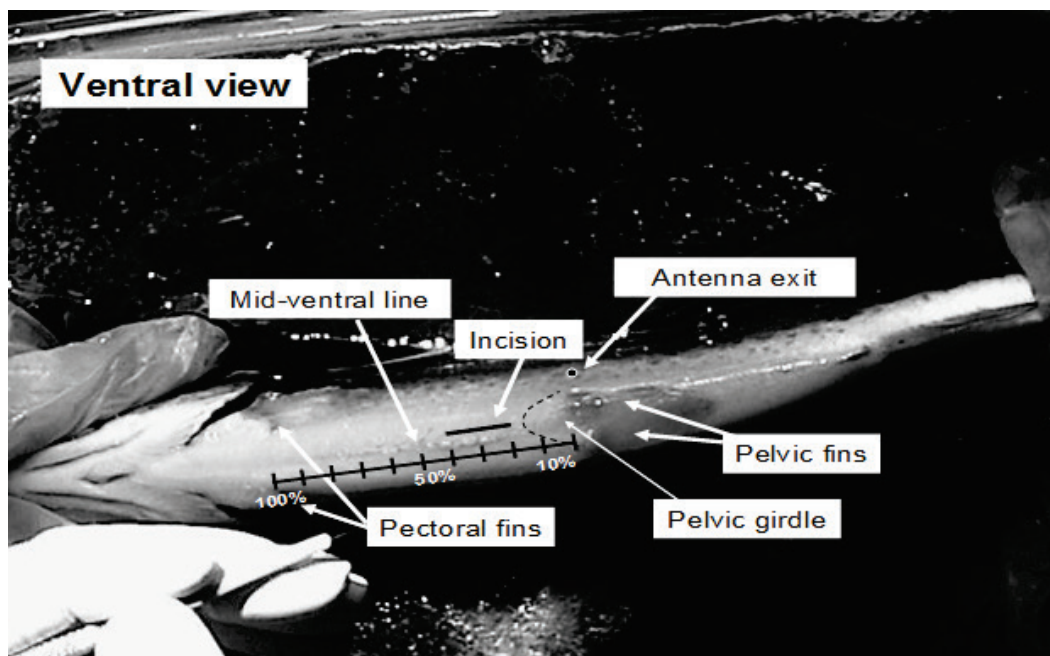


Figure A5. Ventral view of a juvenile salmonid. This shows the location external organs and proper placement of incision and antenna exit (if applicable). Courtesy of Liedtke et al. (2012).



References

- Liedtke, T. L., J. W. Beeman, and L. P. Gee. 2012. A standard operating procedure for the surgical implantation of transmitters in juvenile salmonids: U.S. Geological Survey Open-File Report 2012-1267.

Appendix B: Tagging Late-Fall-Run Chinook Salmon at the Coleman National Fish Hatchery

Standard Operating Procedure (SOP)

Adapted from Liedtke et al. (2001), and the 2011 procedures from Cramer Fish Sciences, the SOPs used for the Department of Interior's South Delta telemetric studies, and the 2011 work instructions used for the U.S. Army Corps of Engineers telemetric studies.

Purpose and scope:

This SOP provides the steps needed to tag hatchery late-fall-run Chinook salmon at the Coleman National Fish Hatchery for the 2015 Fremont Weir Fish Behavior Study. Over the course of two days, 250 late-fall-run Chinook salmon will be tagged at the hatchery and held in circulars until the salmon are ready for release. At a minimum, the following staff will be required to implement this SOP:

- Two surgeons to tag salmon and to work on equipment setup,
- Two data recorders to help with recording data in a Microsoft Access database and to help prepare the acoustic tags, and
- Two fish runners to help with moving tagged Chinook salmon to the circulars, preparing recovery buckets, and taking water quality data.

When applicable, this SOP identifies the tasks that are assigned to the data recorders and fish runners. Any tasks not assigned to these staff are directed to the surgeons. However, the surgeons can seek assistance from the data recorders and fish runners when appropriate.

Materials:

1. YSI ODO dissolved oxygen meter
2. Hardness (CaCO₃) water quality test kit
3. Thermometer for quick temperature checks
4. pH meter or litmus paper
5. Acoustic tags (V-4)

6. VEMCO acoustic tag activator with manual. Manual found at http://vemco.com/wp-content/uploads/2013/03/vta_manual.pdf.
7. VEMCO acoustic tag verification equipment (VR-100 with 180 VH hydrophone) with manual. Manual found at http://vemco.com/wp-content/uploads/2013/02/vr100hw_manual.pdf.
8. Distilled or de-ionized water (D-H₂O)
9. Chlorhexidine solution (Nolvasan ; 30 mL/L D-H₂O)
10. Aquí-S 20E (10% eugenol)
11. Stress coat - stock concentration and 25% solution (250mL/L D- H₂O)
12. Disinfectant solution (i.e., 70% ETOH)
13. PVP iodine (Ovadine)
14. 19 L black bucket(s) marked at 10 L and clearly labeled “Anesthesia”
15. 19 L buckets for post-surgical recovery of fish and for rejecting fish
 - a. Liedtke et al. (2012) does not recommend the use of white or black buckets. White buckets are not ideal for restricting light penetration, while black buckets are too dark and absorbs large amounts of solar radiation. However, the color of the bucket will not be an issue for this study since tagging will either be conducted indoors or under a canopy for shading.
16. Cooler for storing fish before tagging
17. Two large water containers for surgical stations
18. Water pumps with extension cord and rubber tubing with in-line shut-off valve and terminal narrowing
19. Rubber tubing to return water from drain tray to maintenance anesthetic bath
20. Designated syringes (5 mL) for measuring anesthetic and stress coat
21. Oxygen delivery system (cylinder, regulator, airline, air diffusers) for recovery buckets
22. Fish nets (e.g., sanctuary nets, dips)
23. Nitrile gloves (in all sizes)
24. Scale measuring to the nearest 0.01 g (weighing fish and tags)
25. Large sponges
26. Measuring board with ruler to the nearest mm
27. Surgical platform (cradle)
28. Trays for holding solutions used to disinfect surgical tools
29. Trays to rinse disinfected tools
30. Needle drivers (multiple sets)
31. Forceps (multiple sets)
32. Scalpel handle and blades (multiple sets)
33. Scissors (multiple sets)

34. Tissue collection supplies: scissors, blotter paper, labeled coin envelopes
35. Sutures: 19 mm 3/8 circle needle with 4/0 Mono-Dox (violet monofilament polydioxanone) suture material
36. Spray bottles for disinfectant solution
37. Timers and stopwatches
38. Sharps container
39. Datasheets, clipboards, and writing tools
40. Three laptops for data recording
41. Carabiner tag labels to identify fish in recovery buckets
42. Clean rags for keeping tagging areas clean and dry
43. Tables and chairs

Pre-tagging Activities:

- Prior to the tag implantation, the tagging coordinator will need to get in touch with the Coleman National Fish Hatchery on the following items:
 - Notify hatchery staff on the pre-tag fish-holding period requirements. The pre-tag fish-holding period should be 18 to 36 hr.
 - Food should be withheld during the pre-tagging holding period.
 - Notify hatchery staff on the list of study personnel that will be at the hatchery and tagging schedule. All study personnel must bring government issued identification, such as a California driver's license.
- Disinfect all buckets and coolers with PVP iodine (e.g., Ovadine) either at the hatchery or prior to arriving. If this step is not completed prior to arrival, then all equipment must be disinfected at the hatchery before use.

Equipment Setup:

- *Datasheet Setup*
 - Start the electronic *Tagging Datasheet* in a Microsoft Access database for each tagging station. Each data recorder should have a separate database.
 - Prepare a hard copy *Daily Fish Reject Tally Datasheet* for each tagging station to account for fish that are handled, but are not used for the study.

- *Tag Activation*
 - All tags will have been activated prior to tagging.
 - The data recorder will sterilize the acoustic tag in a solution of Nolvasan for a few minutes. Following disinfection, thoroughly rinse transmitters in distilled or deionized water prior to implantation.
 - The data recorder will record the tag serial ID, the tag code ID, and the surgeon and data recorders name in the electronic *Tagging Datasheet* after tag verification.
 - Calibrate the scale and weigh one tag to the nearest 0.01 g in the *Tagging Datasheet* to verify the tag weight and determine minimum weight requirements for tagged fish.
- *Setting up Circulars*
 - Check to make sure that the six circulars for tagged Chinook salmon have water circulating through them. Afterwards, label each circular with a study circular ID with white duct tape and a Sharpie pen.
 - Circular 1,2,4, and 5 will contain 50 tagged fish each.
 - Circular 3 and 6 will contain 25 tagged fish each.
- *Filling and Preparing Trays and Buckets*
 - Fill disinfection trays for surgical instruments with diluted Nolvasan.
 - Fill rinse trays with de-ionized or distilled water.
 - See Figure 1 for example of tray setup.
 - Clip on numerical tag labels to recovery buckets, which will serve as the bucket ID.
- *Water Temperature Checks for Anesthesia Bucket, Surgical Bath, and Holding Cooler*
 - Water temperatures during all aspects of the tagging operations cannot exceed 2°C difference from the reference water source. The fish runners or surgeons will check all water sources periodically and record results in the *Tagging Datasheet* to ensure that water temperature levels are within criteria. For this study, the rectangular tank where source fish are held is the reference water source.
 - Anesthesia buckets, maintenance bath containers, and recovery buckets should not be filled until near the time that they are needed to avoid warming.
 - Anesthesia buckets and maintenance bath containers should be replaced regularly to prevent increasing water temperatures over time.
- *Equipment Setup for Recovery/Reject Buckets*
 - Set up the oxygen cylinder with a trigger.
 - The oxygen cylinder will be used for the recov-

ery/reject buckets. Prior to use, conduct an experiment to see how long you should hold the trigger to target a DO saturation of 120 to 150% in the recovery bucket. Seven sec was used in past SOPs, but this may differ for this study.

- This recovery bucket should be attached with an air stone and air pump.
- *Euthanasia*
 - Set up a separate bucket for any necessary euthanasia. An Aqui-S solution of 175 mg/L should be used for 20 min to euthanize any fish that cannot be returned to the raceway.

Fish Selection Criteria:

- For all experimental groups, handling protocols will be standardized to reduce potential bias (i.e., fish length, number of times handled, tagging procedures, transport methods, transport time, and release protocol).
- VEMCO V4 tags weigh about 0.41 g in the air. The estimated minimum length and weight of the Chinook salmon for surgical tagging should be >8.2 g (tag weight ≤ 5 % of the body weight), respectively. Fish should be targeted between 90 – 100 mm in FL.

Fish Tagging:

- *Equipment setup*
 - Prepare surgical table and equipment for use.
 - The surgeon should wear clean gloves during all procedures that involve handling fish.
 - The surgical station will be cleaned and wiped down with a solution of disinfectant, and surgical instruments will be placed in a disinfectant bath (e.g., dilute Nolvasan, chlorhexidine solution) before fish handling and surgical procedures.
 - Surgical instruments will be transferred to a freshwater rinse bath before surgery and rinsed twice.
 - Rinse tray should be changed often to avoid accumulation of disinfectant in rinse water.
 - To minimize the chances for pathogen transfer between fish populations, all equipment used for capture, holding, anesthesia, surgery, recovery, and movement of

- fish during the project will be thoroughly cleaned and disinfected before use with a different fish population.
 - Soiled gloves should be changed immediately and after handling 10 fish.
 - Set up measuring board and scale. A sponge should be placed on the scale when weighing the fish to reduce the stress to fish and for the ease of handling.
 - Put approximately 1-2 mL of diluted stress coat on the weigh sponge and the measuring board.
 - For each tagging station, the fish runner must fill a 19 L bucket halfway with circular water. In addition, the bucket should be supplied with a small amount of undiluted stress coat, with oxygen using an oxygen cylinder. The bucket should also be fitted with an air stone/air pump before tagging.
 - The concentration of DO in the recovery buckets should be between 120% and 150% saturation by holding the trigger for a few sec.
 - These 19 L buckets serve both as the recovery bucket and the reject bucket. The bucket becomes the recovery bucket if the fish was tagged and it becomes the reject the bucket if the fish was handled, but not used for the study.
 - Fish rejected during surgery can not be returned to the raceway and will be euthanized in a concentrated solution of Aqui-S (175mg/L).
- *Administration of Anesthetic*
 - The effectiveness of Aqui-S as an anesthetic varies with factors, such as temperature, fish density, and individual sensitivity. Adjustments of the anesthesia concentration should be based on the amount of time it takes for a fish to lose equilibrium. Any adjustments should be recorded in the *Tagging Datasheet* with a separate treatment ID.
 - Fill the anesthesia bucket with 3 gallons of circular water. As a suggestion for a starting concentration, add 3.4 mL of Aqui-S to the water using a syringe. This will yield an anesthetic concentration of 30 mg/L. Base the daily starting concentration on fish responses during the tagging operation from the previous days. Rinse the syringe with treatment water to ensure all Aqui-S is dispensed.
 - Aqui-S should be added directly, while constantly mixing, to the full volume of treatment water. Do not make stock solutions or any other dilute solutions of Aqui-S prior to use.
 - Prepare the maintenance bath containers with water from the

circular and with a water pump/tubing (see Figure 2 for setup). This is done by completing the following procedures:

- Fill the container with 10 gallons of circular water.
 - Place the pump into water and ensure tubing is fit properly.
 - As a suggestion for a starting concentration, add 5.7 mL of Aqui-S to the water using a syringe. This will yield an anesthetic concentration of 15 mg/L. Base the daily starting concentration on fish responses during the tagging operation from the previous days. Rinse the syringe with treatment water to ensure all Aqui-S is dispensed.
 - Water in all containers (anesthesia and maintenance) should be changed regularly to minimize dilution of anesthesia water and temperature changes. Moreover, this is done to ensure you do not run out of water during a procedure.
 - Add a small amount of diluted stress coat for each liter of water in the anesthesia and maintenance bath to protect fish from loss/damage to the slime layer.
- *Anesthetizing the Fish*
 - With help from the hatchery, identify the proper raceway containing fish that will be used for the study and place a subset of those fish into a cooler with an air pump and air stone.
 - Having fish in a cooler will help the surgeons gather fish for tagging and avoid a long commute from the raceway to the tagging station.
 - Use a sanctuary net or dip net to remove one fish from the pre-tag holding cooler and place directly into an anesthesia bucket.
 - Remove the fish from the net by hand, taking care not to dilute the anesthesia bath with water from the net.
 - Note: The most significant source of stress that fish experience is usually from being netted. Every effort should be made to minimize handling and sanctuary nets should be used when feasible.
 - Secure the lid as soon as the fish is in the bucket.
 - Call out “fish in drugs” and start a timer to keep track of how long a fish has been in the anesthesia bucket. The data recorder will record the start time of when the fish was placed in the anesthesia bucket in the electronic *Tagging Datasheet* based on when the surgeon called out “fish in drugs.” Time is recorded in the 12-hour clock notation in the following format: hh:mm:ss am/pm.

- Remove the lid after about 1 minute to observe the fish for loss of equilibrium. Once the fish loses equilibrium, keep the fish in the water for an additional 30 to 60 sec. When you take the fish out of the anesthesia bucket, call out “fish out of drugs.” At that point, the data recorder will record the end time of when the fish was placed in the anesthesia bucket in the “Time out of drugs” column in the electronic *Tagging Datasheet*. Time is recorded in the 12-hour clock notation in the following format: hh:mm:ss am/pm.
 - Relay any information to the data recorder. Time of sedation should normally be 2 to 4 min, with an average of about 3 min. If loss of equilibrium takes less than 1 min or if a fish is in the anesthesia bucket for more than 5 min, then reject that fish. If after sedating a few fish and they are consistently losing equilibrium in more or less time than what is typical, then the anesthesia concentration may need to be adjusted. This should only be done after consultation with the field lead, and should be done in 0.5 mL increments. Concentration changes should be executed for all surgeons simultaneously and recorded on the *Tagging Datasheet*.
- Start a timer when a fish is removed from the anesthesia bucket to document the time the fish is out of the water. Once the fish is out of the anesthesia bucket, measure fish length, weight, and condition for the *Tagging Datasheet* using the steps described below:
 - Transfer the fish to the scale and weigh to the nearest 0.01 g.
 - Transfer the fish to the measuring board and measure fork length (FL) to the nearest mm.
 - Evaluate eye, scale, and fin condition and rate them as “good” (g), “fair” (f), or “poor” (p).
 - If a fish is determined to be unacceptable for tagging prior to surgery, place the fish in the reject bucket and inform the data recorder to update the *Daily Fish Reject Tally Datasheet* and to update the *Tagging Datasheet*.
 - In addition, inform the fish runner to transport the fish in the reject bucket to the reject tank. Fish should be transferred through water-to-water transfers.
 - If the fish is determined to be unacceptable during surgery, the fish will be euthanized.
- Data must be vocally relayed to the data recorder and the data recorder should repeat the information back to the

- surgeon to avoid miscommunication.
- Any fish that is dropped on the floor during this process must be rejected.
 - A fish dropped on the table during surgery may still be tagged.
 - If a fish is dropped on the floor after it is tagged, then remove the tag and reject the fish.
 - The data recorder should document this information in the *Daily Fish Reject Tally Datasheet* and update the *Tagging Datasheet*.
 - *Implanting a Surgical Tag*
 - Selected fish will be bathed in cool (<14 °C), aerated water during surgery. Surgery will be performed in as sterile an environment as possible.
 - Fish will be placed ventral-side up on a surgery cradle made of Microcell foam with a size-specific mold to hold the fish in position.
 - See Figure 3 to 5 at the end of this SOP for general reference of surgical procedures.
 - Water diffused with a maintenance anesthesia solution (15 mg/L) will be passed through the tubing using a pump and will continually flow into a reservoir in the mold where the fish's head will be submerged. This will gently flush the anesthetic solution over the gill membranes to ensure oxygen and anesthesia is carried to, and metabolic wastes are efficiently moved away from, the gills continuously throughout the procedure. Using the in-line valve, adjust the flow as needed, so that the gilling rate of the fish is steady.
 - Using a Sharp point 15° stab point (3.0 mm or 5.0 mm) restricted blade depth scalpel, an approximate 5 mm incision will be made parallel to and 2 mm to the side of the ventral midline and anterior to the pelvic girdle.
 - One scalpel blade can be used on 5 to 7 fish before it becomes dull. If the blade is pulling roughly or making jagged incisions, it needs to be changed.
 - Use blunt tipped forceps or hemostat to open the incision to ensure you did not damage any internal organs or cause excessive bleeding.
 - Do not implant the tag and reject that fish if you observe damage or think you damaged an organ. Excessive bleeding indicates likely organ damage. Therefore, it should be noted on the *Tagging Datasheet* if the surgery continues.
 - In order to avoid cutting into the pelvic girdle with the scalpel incision, consider making the incision from the tail towards the head. This will reduce the chance of

- tearing skin near the pelvic girdle. Even a small nick in the pelvic girdle will compromise swimming ability.
- A disinfected tag will be inserted through the incision into the peritoneal cavity of the fish. Tags should only be handled by gloved hands or clean surgical instruments such as forceps after the disinfection step.
 - The tag will be positioned, so it is lying immediately under the incision.
 - If a battery side is evident on the tag, it should be inserted first with the battery oriented parallel to the in. As the tag is placed into the peritoneal cavity, the battery should be pushed towards the tail and the transducer of the tag should be towards the head.
 - This positioning will provide a barrier between the suture needle and internal organs. Through time, the tag location will naturally move posterior in the fish.
 - The incision will be closed with one simple suture using the 10.5 mm (NP-1) precision point, 3/8 circle needle with 4/0 Mono-Dox (violet monofilament polydioxanone) suture material.
 - Note: While suturing in and out, forceps should be used to separate the skin from muscle and organs to avoid suturing anything but the skin.
 - To make a stitch, lock the needle (at the end of the suture) in the hemostat so the needlepoint faces you. Enter the outside edge of the incision on the side farthest from you and exit through the other edge of the incision, pulling the suture perpendicular through the two edges. The needle should enter and exit the skin as close to the edge of the incision as possible without tearing the skin (~ 2 mm from edge of incision).
 - Pull the needle and suture through the skin to leave a tag end of about 2 to 3 cm of suture material protruding from the needle entrance location. Afterwards, release the needle from the needle drivers.
 - With your non-dominant hand, grasp the long end of the suture material (usually with thumb and forefinger) at or below the needle, and make two forward wraps (i.e., away from your body) around the tip of the needle driver, which should be held in your dominant hand.
 - With the two wraps still around the needle driver, grasp the short tag end of suture material with the needle driver and tighten the stitch by pulling the wraps off the needle driver and pulling both ends of suture material perpendicular to the incision.

- On the first knot, the dominant hand holding the needle driver should pull toward your body and the non-dominant hand should pull away from your body. Tighten the suture lightly, just so the edges of the incision meet, but do not overlap, pucker, or bulge the edges of the incision. The second knot is the same as the first, but in reverse order.
- On the second knot, grasp the long end of suture material with your non-dominant hand, make two reverse wraps (i.e., toward your body) around the end of the needle driver, grasp the short end of suture with the needle driver, and tighten the stitch. This time, the knot should be tightened by pulling your dominant hand (holding the needle drivers) away from you and your non-dominant hand toward you. The second knot can be slightly tighter than the first, again taking care not to overlap, pucker, or bulge the edges of the incision. This completes one knot.
- Cut the suture with the hemostat or scissors, leaving ends approximately 2 mm in length.
- If the incision is too long to close with one stitch, it is acceptable to add a second suture knot. Relay this information to the data recorder to record in the “Notes” section of the *Tagging Datasheet*. Furthermore, the surgeon will tell the data recorder if the incision, suturing, and tag placement was “good” (g), “fair” (f), or “poor” (p). Lastly, the surgeon should determine the level of bleeding (0, 1, 2, 3).
 - If the fish is in bad condition, then the fish should be rejected.
- Call out “surgery complete” and transfer the fish from the surgical platform to the appropriate recovery bucket for ten minutes. This should be done with minimal handling by moving the platform as close as possible to the bucket or using a liner material to lift the fish for transfer.
 - After the surgeon calls out “surgery complete,” the fish runner should start the timer for ten minutes and the data recorder should record the actual time in the “Time out of Surgery” column of the *Tagging Datasheet*. Actual time should be recorded in the 12-hour clock notation (hh:mm:ss am/pm). In addition, there should be one fish per recovery bucket.
 - When ten minutes is up, the fish runner will transport the fish to the circular (see next section).
 - Each individual suture (one packet) can be used on approximately five fish. Disinfect the suture material and

the attached suture needle in the sanitizing solution used for instruments.

- Between surgeries, the surgeon should replace the tools that were just used into the disinfectant bath. Each surgeon will have at least 3 sets of surgical instruments to rotate through to ensure that tools get a thorough soaking in disinfectant between uses. Each surgery station will have one tray of Nolvasan, one tray of diluted Nolvasan, and one of distilled or de-ionized water.
 - Once disinfected in Nolvasan solution, rinse the tools thoroughly with distilled or de-ionized water and ensure that the scalpel blade and suture are ready to use on the next fish. Organic debris in the disinfectant bath reduces its effectiveness, so be sure to change the bath regularly. If necessary, replace the scalpel blade.

Placing Tagged Fish into the Circulars:

- After the fish has stayed in the recovery bucket for ten minutes, the fish runner should remove the lid and make sure the fish has “recovered.” This means that the fish has regained orientation and is maintaining upright swimming.
 - If the fish is no longer alive, then the fish runner should bring back the fish in the bucket to the surgeon. Afterwards, the surgeon will perform a necropsy and retrieve the tag (see *Performing a Necropsy of Tagged Chinook Salmon* SOP for procedures).
 - If the fish recovered, then the fish runner will move the bucket and clipboard over to the circular for holding the fish.
 - Once at the circular, the fish runner should release the fish into the tank. This is done by partially submerging the bottom of the recovery bucket into the circular and gently tilting the bucket until the fish is released into the circular.
 - When the fish is released, the fish runner will call out “recovery complete” with the bucket ID. Afterwards, the data recorder will record the time in the 12-hour clock notation in the “Recovery” column of the *Tagging Datasheet*.
- It is the responsibility of the fish runners to keep track of the number of fish that are placed into a circular. This should be done through tallies on a field notebook.
- When there are 25 or 50 fish in the circular, the tagging coordinator should record the total number of fish in the tank and document any

mortality on a field notebook. Any dead salmon should be bagged in separate Ziploc bags and labeled with the date of bagging, the time of bagging using the 24-hour clock notation (hh:mm), and the circular in which the dead salmon was found. Afterwards, the surgeon will perform a necropsy and retrieve the tag (see *Performing a Necropsy of Tagged Winter-run Chinook Salmon* SOP for procedures).

- After 25 or 50 fish are placed into a single circular, the fish runner should move on to the next circular using similar procedures. Three circulars should be filled per day (two with 50 fish and one with 25 fish).
- At the end of each day, the fish runner or data recorder should record the number of fish and total fish weight in each cooler. This information should be given to the hatchery and is used to determine the rough amount of feed to place into each tank.
 - Water quality will be taken through out the day and used to ensure conditions in the holding tanks remain acceptable.

End of Session Activities:

- Validate the tag data and datasheet accuracy.
 - Working together, each tagger and assistant team will review the transmitter tubes/serial numbers against the *Tagging Datasheet* to verify that all of the transmitters provided for the session were implanted into study fish. The steps of the verification process should include reading the serial number on each tag tube, finding that serial number on the datasheet to confirm that it was implanted.
- Export the Access datasheets to Excel and review the information on the *Tagging Datasheet* and complete any missing information.
- Organize tagging solutions and surgical instruments to be ready for the next tagging session.
- Provide the Coleman National Fish Hatchery with any datasheets that they need for the fish care process. This includes all of the datasheets needed for the fish care process and the necropsies that have to be performed in case there are morts.

End-of-Day Clean up:

- At the end of each tagging day, wipe down or spray all surfaces with 70% ETOH to disinfect.
- Move rejected fish back into the circulars and dispose of any euthanized fish based on the protocols by the hatchery.

- Use a toothbrush to remove all large organic debris from instruments, rinse them and dry them to prevent rust.
- Make surgical tagging solutions as needed to be ready for the next tagging session.
- Inventory chemical solutions and tagging supplies (blades and suture).
- Return any soiled rags to the office and have them washed.
- Rinse buckets with hose and place upside down to dry.
- Turn off the oxygen cylinder.

General Fish Handling Reminders:

- Anesthesia and freshwater containers and buckets should be filled just prior to tagging to avoid temperature changes and should be changed often. Check levels of carboys before each surgery to be certain that you will not run out of water during a surgery.
- **USE CAUTION and COMMUNICATION** when adding Aquí-S to any container to avoid adding two doses or no doses to the container.
- Keep a lid on any bucket or cooler that contains fish.
- Any fish dropped on the floor should be rejected. If a fish is dropped on the floor after it has been tagged, then remove the tag, and place it into another fish. The dropped fish will then be euthanized.
- **CAREFULLY HANDLE BUCKETS.** Try not to bang them around, slam the handles, or otherwise handle in a rough manner as this can stress fish.
- **USE A SANCTUARY NET** to capture source fish and place them into an anesthesia bucket. A recommended approach is to use a non-sanctuary net in the container of source fish in order to be able to capture the fish without them detecting the pressure wave in front of the sanctuary net. Once a fish is in the traditional net, place the sanctuary net immediately below the fish so that the handles of the two nets are aligned and can be handled together.

Figures

Figure B1. Example of setup for disinfecting and rinsing surgical equipment. The figure shows one container of Nolvasan, one contained of diluted Nolvasan, and one container of distilled water.



Figure B2. Example of setup of maintenance bath (large container) with drain tray (smaller container) and surgical platform.

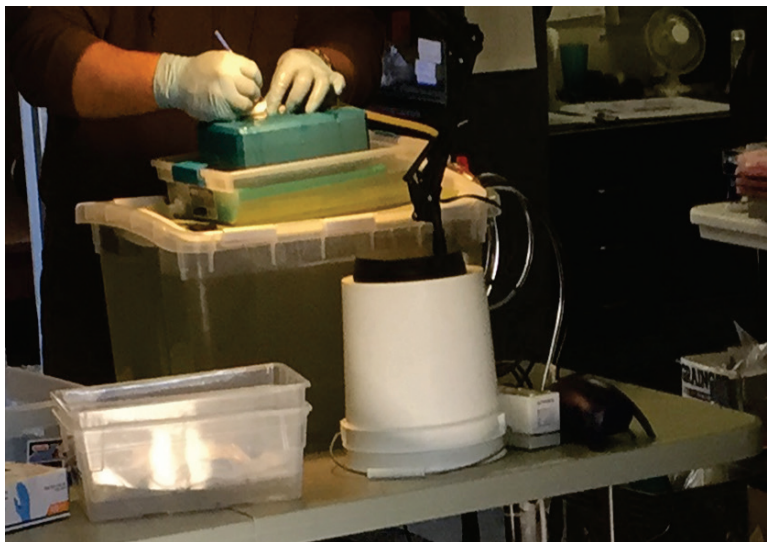


Figure B3. Lateral view of a juvenile salmonid, showing the location of internal organs. Courtesy of Liedtke et al. (2012).

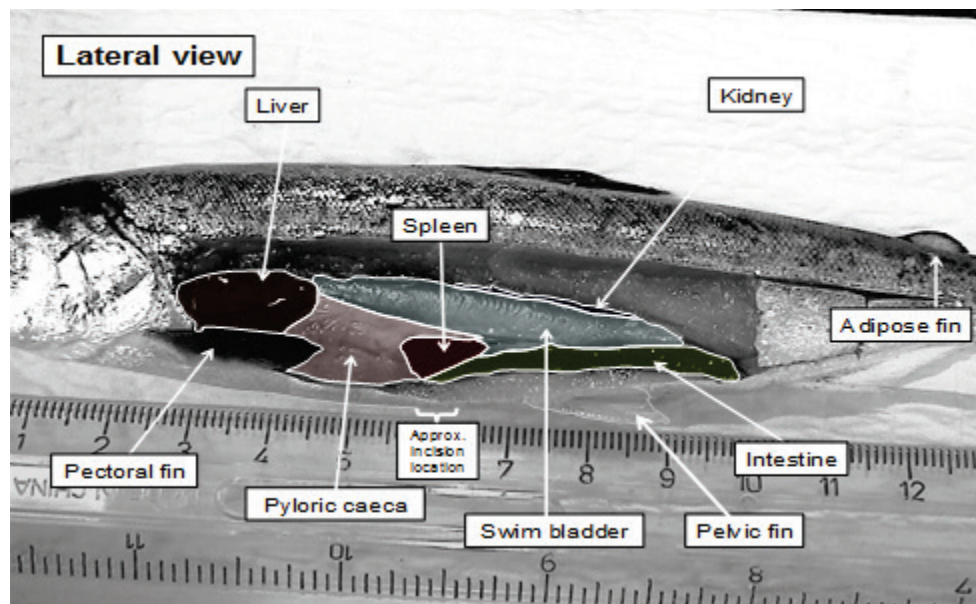
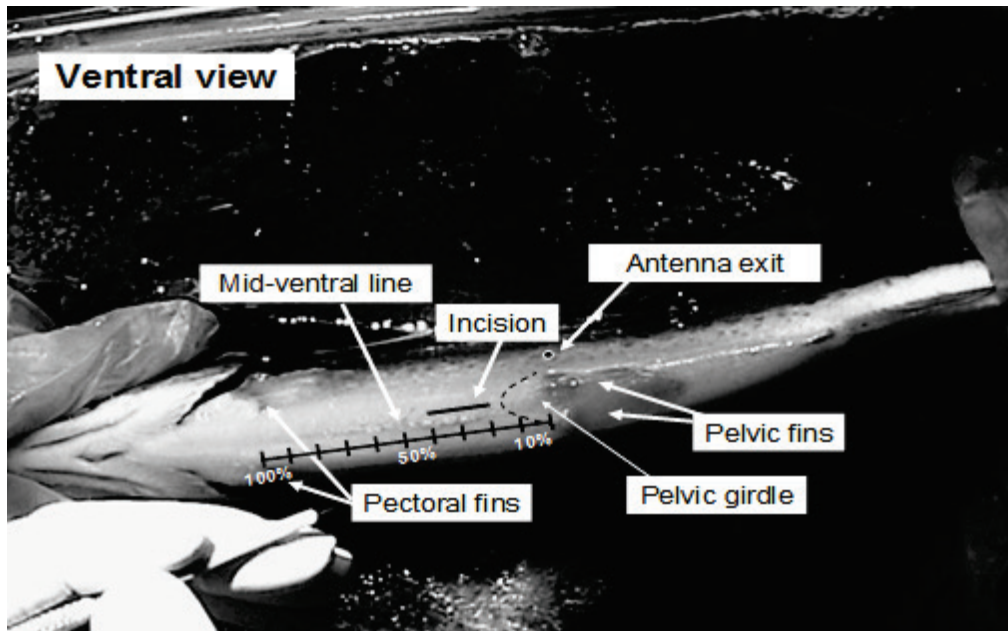


Figure B4. Proper surgical procedures help insure rapid recovery and incision healing (note proper incision healing on photo right). Courtesy of Cramer Fish Sciences.



Figure B5. Ventral view of a juvenile salmonid. This shows the location external organs and proper placement of incision and antenna exit (if applicable). Courtesy of Liedtke et al. (2012).



References

- Liedtke, T. L., J. W. Beeman, and L. P. Gee. 2012. A standard operating procedure for the surgical implantation of transmitters in juvenile salmonids: U.S. Geological Survey Open-File Report 2012-1267.

Daily Reject Tally Datasheet

2015 Fremont Weir Fish Behavior Study

Tag Date: _____ Surgeon: _____
Data Recorder: _____

Species (circle one): **WCHN** or **LFCHN**

<u>REJECTS</u>	<u>TALLY</u>
Disease	_____
Descaling	_____
Dropped	_____
Injury	_____
Fungus	_____
Anesthesia	_____
Too small	_____
Too large	_____
Specify Other	_____
Specify Other	_____
Specify Other	_____
Specify Other	_____
Specify Other	_____
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Specify Other	_____
Specify Other	_____
Total:	<div></div>

Tagging Datasheet

2015 Fremont Weir Fish Behavior Study (Late-fall-run Chinook Salmon)

Background: The *Tagging Datasheet* is entered electronically in a Microsoft Access database. The following fields need to be created for this datasheet.

Field	Description
Date Tagged	Enter as mm/dd/yyyy.
Date Released	Enter as mm/dd/yyyy. This data is entered after fish are released.
Time Released	This data is entered after fish are released.
Treatment ID	Used to identify the concentration of Aqui-S used in the surgical bath and anesthesia bucket. The use of A and B is used to identify the surgeon.
Study ID	Used to ID the study. All records should be recorded as FY 15 Tracking.
Fish ID	Used to identify the fish for the study. Recorded as LFC ###.
Species	All records should be LFC (late-fall-run Chinook)
Tag SN	Serial number of the V4 tag
Tag ID	Enter tag code ID
Tank	Enter the circularID where fish was placed after tagging.
Bucket	Enter the bucket ID for the fish, which is used to identify the fish during the tagging process. This number is not unique for every fish.
Cooler	The cooler that the fish was placed into during the transport process.
Tag Type	All should be V4
Weight (g)	Enter the weight, measured to the nearest 0.01 g
FL (mm)	Enter the fork length (FL), measured to the nearest whole fish
Time in Drugs	Recorded in 12-hour clock notation
Time out of Drugs	Recorded in 12-hour clock notation
Time out of Surgery	Recorded in 12-hour clock notation

Recovery Time	Recorded in 12-hour clock notation
Fins	Entered as “good” (g), “fair” (f) or “poor” (p)
Scales	Entered as “good” (g), “fair” (f) or “poor” (p)
Eyes	Entered as “good” (g), “fair” (f) or “poor” (p)
Parr Marks	Entered as “yes” (y) or “no” (n)
Incision	Entered as “good” (g), “fair” (f) or “poor” (p)
Tag Placement	Entered as “good” (g), “fair” (f) or “poor” (p)
Suture	Entered as “good” (g), “fair” (f) or “poor” (p)
Bleeding	Evaluated as 0,1,2,3
Tag Tested	Enter Y (yes) or N (no) if tag is verified with VR-100.
Tag Weight (g)	Measured to the nearest 0.01 g.
Surgeon	Enter the name of surgeon.
Data Recorder	Enter the name of the data recorder.
Days starved	Enter the amount of days the fish were starved.
Surgical Temp (°F)	Enter water quality data as measured in the surgical bath.
Surgical pH	
Surgical DO (mg/L)	
KO Temp (°F)	Enter water quality data as measured in the anesthesia (knock out) bucket.
KO pH	
KO DO (mg/L)	
Holding Temp (°F)	Enter water quality data as measured in the holding cooler.
Holding pH	
Holding DO (mg/L)	
Notes	Anything interesting of note that could impact the results.

Appendix C: Daily Fish Care after Tagging at the Livingston Stone National Fish Hatchery

Standard Operating Procedure (SOP)

Adapted from Afentoulis et al. (2014).

Purpose and Scope:

The following procedures and guidelines shall be implemented by Fish and Wildlife Service (FWS) staff to maintain the health of all acoustically tagged winter-run Chinook salmon at the Livingston Stone National Fish Hatchery. This will have to be done when salmon are held at the hatchery after tagging for the Fremont Weir Fish Behavior Study. These salmon will be held at the hatchery until they are ready for release. In total, 250 winter-run Chinook salmon will be tagged and these salmon will be placed in circulars that are inside the hatchery in groups of 25 fish. These circulars operate as a flow through system and should be covered with a screen. In addition, each circular should be clearly labeled with white duct tape for the study.

All data collected during the fish care process will be recorded on the *Daily Feed Log* and the *Daily Water Quality after Tagging Log*. These logs will be stored in a binder or folder with the hatchery coordinator. The “General” section of the *Daily Feed Log* should be filled out by the tagging crew with the average weight of salmon, the average fork length of salmon and the exact number of salmon in each circular. If this is not the case, then please contact the tagging coordinator.

Points of Contact:

Any reference to the tagging coordinator or the hatchery coordinator in this SOP refers to the following staff. Please contact the lead contact before the back up.

Tagging Coordinator

Role	Name	E-mail	Office Phone	Cell Phone
Lead Contact	Josh Israel	jaisrael@usbr.gov	916-414-2417	916-296-8702
Back Up	Jason Hassrick	jhassrick@usbr.gov	916-414-2416	916-425-9121

Hatchery Coordinator

Role	Name	E-mail	Office Phone	Cell Phone
Lead Contact	John Rueth	john_rueth@fws.gov	530-275-0510	

Materials:

1. Daily Water Quality after Tagging Log from the hatchery coordinator
2. Daily Feed Log from the hatchery coordinator
3. Dissolved oxygen and water temperature meter
4. Feed and feeder
5. Manufacture feed chart
6. Tank mort net
7. Ziploc bags
8. Sharpie marker
9. Calculator

With the exception of datasheets, all materials are provided by the hatchery. The tagging crew will provide the hatchery coordinator with the appropriate datasheets.

Daily Tasks:

The tasks below are essential for maintaining salmon health, so that the salmon are in optimal conditions when they are released into the Sacramento River. Note that not all tasks may need to be performed each day. Tasks that do not have to be completed daily are described below.

A. Water flow checks

1. Check that there is water flow into and out of the circular before and after fish care. This is just a cautionary step. There should

never be any issues with not having water flow into and out of each circular.

B. Mortality counting and removal

1. On a daily basis, visually scan each circular for dead salmon and remove any dead salmon with the tank mort net. Document any morts in the comments section of the *Daily Feed Log* (e.g., “one mort in circular #1 on 1/14/1900 at 1700 hours”).
2. Place morts in a plastic Ziploc bag and label it with a Sharpie marker with the date and time (24-hour clock notation, hh:mm) of bagging, and the circular ID from the study. Each salmon should have its own plastic bag. Afterwards, perform a necropsy and retrieve the acoustic tag using the *Performing a Necropsy of Acoustically Tagged Chinook Salmon* SOP. All retrieved tags should be placed in its own-labeled Ziploc bag and returned to the hatchery coordinator. The hatchery coordinator will provide the retrieved tags to the transport crew who will return the tags to the tagging coordinator.

C. Dissolved oxygen and temperature checks

1. Any meters used should be calibrated as necessary.
2. On a daily basis, measure dissolved oxygen (mg/L and % saturation) and water temperature (°C) in a circular. One circular should be measured each day. The circular that needs to be measured each day is provided in the *Daily Water Quality after Tagging Log*.
3. Record your measurements in the appropriate columns in the *Daily Water Quality after Tagging Log*.

D. Circular cleaning

1. Cleaning of the circulars should occur when needed to remove accumulated debris.

E. Feeding

1. Salmon should be fed no later than 0900 hours each day. Feeding should occur daily after 24 hr of tagging, except for the last day of holding. Plan arrival times and fish care activities accordingly.
2. To determine the amount of feed per day, please follow the following procedures:
 - i. Using the manufacturer’s feed chart, locate the appropriate size range column along the top of the table.
 - ii. Find the temperature column on the far right of the table that corresponds with the temperature of the circulars. If the exact temperature is not on the table, then round down to the next temperature.
 - iii. The box where these two columns intersect is the % of fish biomass to feed per day.
 - iv. Multiply the number of fish in the circular by the average weight to get the biomass.

- v. Multiply the biomass by the % biomass number from the table to determine the daily feed ration.
 - vi. Remember this ration is only used as a guideline. Other factors may come into play when deciding on the feed ration, such as feeding methods, water quality conditions, and fish feeding behavior.
 - vii. Weigh out an appropriate amount of feed into the designated feed cup. Record the amount of feed per day in the *Daily Feed Log*.
- 3. Pull the feeder belt back and pour feed onto the feeder. Feed should be distributed onto the feeder so that it falls during day light hours.
 - 4. Sweep up any spilled food near the circulars or the feed station.
- F. Equipment cleaning and disinfection*
- 1. All equipment should be cleaned following the current protocols at the Livingston Stone National Fish Hatchery.

References

- Afentoulis, V., B. Kozak, J. Miranda. 2014. Fish Holding Procedures. California Department of Water Resources, Bay-Delta Office. Sacramento, CA. November 2014.

Appendix D: Transporting, Holding, and Releasing Acoustically Tagged Chinook Salmon

Standard Operating Procedure (SOP)

Adapted from the SOPs used for Department of Interior's south Delta telemetric studies, the 2011 work instructions used for the Army Corps of Engineers' telemetric studies, and the recommendations by Gabe Singer (UC Davis Biotelemetry Lab).

Purpose and Scope:

On January 27, 2015, and February 2, 2015, a transport crew will head down to the Livingston Stone National Fish Hatchery or the Coleman National Fish Hatchery to load and transport 125 tagged Chinook salmon of each salmon run for the 2015 Fremont Weir Fish Behavior Study. These salmon will be transported to Jerry Rose's dock in Knights Landing, California, and placed into holding pens at the dock for at least 24 hours before release.

This SOP describes the steps needed to transport, hold, and release acoustically tagged Chinook salmon into the Sacramento River.

Materials:

- Transport Crew
 - YSI ProODO for dissolved oxygen and water temperature measurements
 - Manual available at <http://www.ysi.com/media/pdfs/626279-YSI-ProODO-User-Manual-RevC.pdf>
 - YSI Pro1030 for salinity measurements
 - Manual available at <http://www.ysi.com/media/pdfs/605182A-English-Web.pdf>
 - Datasheets
 - Buckets
 - Coolers
 - Nets
 - Stress Coat
 - Rock Salt

- Air Stone and Air Pump (with extra batteries)
 - HR-180kHz-EXT Receiver
- Holding Pen Setup Crew
 - See SOP #5 (Constructing and Deploying Trash Can Style Holding Pens)

Pre-transport Activities:

- Notify hatchery staff of the study personnel that will be at the hatchery at least 24 hours in advance. For the Livingston Stone National Fish Hatchery, all study personnel must bring government issued identification, such as a California driver's license.
- Disinfect all buckets, coolers, and nets with the proper disinfectant solution before heading down to the hatchery. A rinse station is also available at the hatchery.
- Calibrate the YSI ProODO and YSI Pro1030.
- Make sure all transport vehicles have been rinsed before heading down to the hatchery.
- Label coolers with white duct tape. Ten coolers will be used for each transport day and each cooler should hold no more than 13 fish.
 - Each cooler should be labeled with the cooler ID. The cooler ID is based on the species code (LF for late-fall-run or WC for winter-run), the circular ID#, and a letter designation (a, b, etc.). The letter designation is used to differentiate fish in different coolers from the same circular. This is needed since a circular can contain more than 13 fish.
 - For example, circular #1 at the Livingston Stone National Fish Hatchery should contain 25 winter-run Chinook Salmon. Since only 13 fish should be in a cooler, 13 fish will go into one cooler and 12 fish would go into another cooler. Therefore, the coolers will be labeled as WC 1a and WC 1b.
- Pre-measure the amount of rock salt that should be placed into each cooler to target 3 ppt. This amount of rock salt should be placed into Ziploc bags.
 - To target 3 ppt, add 3 g of rock salt for every liter of water.
 - Salting the water in the transport coolers reduces the external-internal osmotic gradient between the fish and their environment. When fish are stressed, they produce epinephrine, a hormone that increases the gill surface area (Wedemeyer 1996). As a result, stressed salmonids may rapidly diffuse freshwater into the body, which overwhelms osmotic and ionic regulatory controls. Salting helps to prevent ion imbalance due to

this response (Moyle and Cech 2004).

- Load all items into the transport truck.
- Prepare datasheets used for the transport and release process.

Transport

- Drive down to the hatchery.
- Record the arrival time using the 24-hour clock notation (hh:mm) on the *Transport Datasheet*.
- Start dissolving pre-packaged rock salt with water in buckets. This is done to avoid dropping rock salt on salmon when they are in coolers. This step should be done as soon as the crew arrives at the hatchery since it takes time to dissolve rock salt.
 - Afterwards, mix the water and rock salt with about 15 ml of stress coat.
 - Stress coat is a water conditioner and artificial slime coat. Stress coat keeps the mucus layer intact, which is important because it is the fish's primary barrier against disease and infection. In addition, it plays a role in ionic and osmotic balance. The mucus layer is easily lost during the handling and netting process (see Harnish et al. 2011).
- Identify the circulars with fish that will be loaded into coolers for the day. There should be ten circulars in use at the Livingston Stone National Fish Hatchery and six circulars in use at the Coleman National Fish Hatchery.
 - For each transport day, five circulars would be loaded into coolers at the Livingston Stone National Fish Hatchery, and three circulars would be loaded into coolers at the Coleman National Fish Hatchery.
 - At the Livingston Stone National Fish Hatchery, each circular would have 25 salmon.
 - At the Coleman National Fish Hatchery, only circular 3 and 6 would have 25 salmon. The other circulars have 50 salmon.
- Fill the coolers with water from the hatchery and insert air stones with air pumps for each cooler. At this point, coolers should be filled about 1/4 full to avoid injuries to fish and people.
- For a single circular, transfer the appropriate amount of fish into each cooler.
- Load the cooler onto the transport truck and fill the cooler to near capacity with hatchery water.
- Evenly pour dissolved rock salt with stress coat into the cooler.

- Carefully mix the water in the cooler and check for any dead salmon in the cooler. If there are dead salmon, then place the dead salmon in a plastic Ziploc bag and label it with a Sharpie marker with the date/time of bagging (24-hour clock notation, hh:mm), and the cooler ID from the study (e.g., WC 1a). Each salmon should have its own plastic bag. Afterwards, perform a necropsy and retrieve the acoustic tag using the *Performing a Necropsy of Acoustically Tagged Chinook Salmon* SOP. Measure salinity (ppt), dissolved oxygen (mg/L and % saturation), and water temperature (°C) in each cooler.
 - The dissolved oxygen concentration in all holding containers should be around 80–130% saturation (Liedtke et al. 2012).
 - When using the YSI ProODO for dissolved oxygen and water temperature measurements, make sure to make an adjustment for the salinity value by pressing the probe symbol, highlighting salinity on the screen, and pressing enter. Afterwards, use the numeric entry screen to enter the salinity value of water that you will be testing.
 - This is needed since the ability of water to dissolve oxygen decreases when the salinity of water increases.
 - If water quality measurements are taking a substantial amount of time to record, then only take measurements for a few coolers.
- Repeat these steps until all fish have been placed in coolers.
- At the end, the driver should contact the holding pen setup crew and let them know that the transport crew is leaving the hatchery. The driver should let the holding pen setup crew know the temperature range in the coolers. This will allow the holding pen setup crew to assess the need for tempering the coolers at the release site.
- The driver will record the time that the transport crew leaves the hatchery on the *Transport Datasheet* and head down to Jerry Rose's dock.
 - Time should be recorded in the 24-hour clock notation to the nearest minute.
- During the transportation process, make a stop at Granzella's Restaurant in Williams, California (451 6th Street, Williams, California 95987) for a water quality check.
 - Salinity (ppt), water temperature (°C), and dissolved oxygen (mg/L and % saturation) concentrations should be taken again from each cooler near the halfway point to Jerry Rose's dock. Record the data on the *Transport Datasheet* as measured. In addition, check for any dead salmon and follow similar procedures for handling mortalities that were previously described in the SOP.
 - If water quality measurements are taking a substantial amount of time to record, then only take measurements for a few coolers.

Release Site

- Record the time of arrival at Jerry Rose's dock. The release site should already be set up with holding pens and a field crew trailer by the holding pen setup crew.
- Measure salinity (ppt), water temperature (°C), and dissolved oxygen (mg/L and % saturation) concentrations for each cooler in the *Transport Datasheet*. In addition, check for any dead salmon and follow similar procedures that were previously described for handling mortalities.
- Place the customized HR-180kHz-EXT receiver into the cooler to document which fish are in the coolers. Record the tag codes from the HR receiver in a notebook.
- Take the water temperature and dissolved oxygen concentrations at the release site to determine the need for tempering. Record the information on the *Transport Datasheet*.
 - If water temperature in the coolers is within the 2°C difference of the river temperature, then start loading coolers into the holding pens in the river. This should be done by bucketing out water from the coolers for ease of carrying to the dock.
 - If the water temperature in the coolers is different from the river by more than 2°C, then take out a bucket full of water from each cooler and add a bucket of river water to each cooler. Hold fish for a few minutes prior to retaking water temperature in the coolers. If water temperature in the coolers is now within the 2°C difference, then start loading the fish from the coolers.
 - Otherwise, repeat the procedure until the difference is less than 2°C.
- The fish should be transferred to the holding pen with a sanctuary net. Each holding pen should have 25 fish. This means two coolers of fish (one consisting 12 fish and one consisting of 13 fish) should be loaded per pen.
 - Document which coolers go into which holding pen on the *Release Datasheet*.
 - Record the time of loading on the *Release Datasheet* in the 24-hour clock notation.
 - During this process, check for any dead salmon and follow similar procedures for handling mortalities that were previously described in the SOP.
 - In the end, there should be five holding pens for winter-run Chinook salmon and five holding pens for late-fall-run Chinook salmon for each transport day. Each holding pen should have 25 fish and labeled with a laminated sign (e.g., WHP 1 or LHP 1). "WHP" stands for winter-run

Chinook salmon holding pen and “LHP” stands for late-fall-run Chinook salmon holding pen.

- Once all fish have been placed in holding pens, the transport crew should leave a few buckets and coolers for the release crew. The remaining coolers should be returned to Bryte Yard.

Releasing Tagged Fish:

- After fish have been placed into holding pens, there should always be at least two study personnel present at the release site even when releases are not occurring.
 - At a minimum, the study crew should check the holding pens every hour to make sure that all of the cans are in place and all are upright. Also, check to see if there is enough clearance between the bottom of the holding pens and the substrate. If not, the study crew needs to come up with a plan to provide enough clearance.
 - Any interesting observations should be recorded on the back page of the *Holding Pen Water Quality Datasheet*.
 - Dissolved oxygen concentration (mg/L, % saturation) and water temperature (°C) should be measured around every four hours in the area adjacent to the holding pens.
 - All data should be recorded as measured in the *Holding Pen Water Quality Datasheet*.
 - The schedule for water quality checks should also be posted in the field crew trailer.
- Field crew will release fish at the times provided on the schedule posted in the field crew trailer. Releases should occur every five hours after 24 hours of holding.
 - Release crews should wear appropriate field gear. This includes the appropriate outerwear and PFD when on the boat. There should also be a headlamp at night.
 - During release shifts at night, the release crew will consist of three staff: one boat operator, one boat assistant, and one on-shore staff. The boat operator is responsible for bringing the boat to the release site.
- Identify which holding pens with fish are to be released. Each container is equipped with two tethers with two quick-links attached to the main anchor line. Detach the quick-links from the main anchor line and attach to the transport line located near the starboard side gunnel of the release boat. Two holding pens will be released at a time: one with winter-run Chinook salmon and one with late-fall-run Chinook salmon.
 - If releases are occurring at night, make sure there is one other crewmember on shore and observing the release. He/she is on

site to call for help, assist if the boat capsizes or assist with other emergency-type events.

- Once you have attached the transport containers to the vessel, aboard the vessel and start the outboard engine. The outboard is equipped with a key start; make sure that the outboard is in neutral with the throttle set at start. Once the outboard is running, safely engage the shifter into forward or reverse, depending on orientation of the boat. Afterwards, move away from the holding area and into the center of the channel.
- Maintain a slow and steady speed; making sure that the holding pens are not tipping or submerging. If the holding pens appear to be tipping or submerging, then slow down the rate of speed. If the holding pens are hitting the bottom because the river is too shallow, then pull the cans up further in the water column using a rope looped around the holding pen and the cleat on the boat.
- Once the release location has been reached, remove the wing nuts holding the lid of the holding pen in position. Pull the lid off and place into boat. Once the lid is removed, pull the container slowly up; allowing some of the water to drain. **DO NOT COMPLETELY DEWATER THE HOLDING PEN!**
- Observe the fish inside of the container; making sure there are no mortalities. If you observe a dead fish, then remove it as gently as possible from the container and place it into a Ziploc bag. Record the number of mortalities for each holding pen on the *Release Datasheet*. Once you have retrieved any mortalities from the holding pen, slowly invert and push the can down so that one end of the opening is just under the surface of the water. Allow the fish to swim out of the holding pen.
 - If necessary, turn the can upside down to empty the contents of the container into the river. Make sure that all fish have left the container prior to bringing the container on board the boat. Once the container is empty, place it inside of the boat.
- Using the atomic clock, record the date and actual time of release (to the nearest minute in 24-hour time) on the *Release Datasheet* for each holding pen.
 - Do not write down the time from the schedule if this is not the actual time of release. Also, remember to change the date if the release is after midnight.
- On the boat, measure dissolved oxygen concentration (mg/L, % saturation) and water temperature (°C) after the release.
 - All data should be recorded as measured in the *Release Datasheet*.
- Repeat the procedure for the remaining holding pens. Make sure that you record release date and time for each group of fish. Return to shore.

- Remove the empty holding pens from the vessel and place on shore. Make sure that the holding pens are placed upside down (frame facing ground). This ensures that the holding pens are not damaged. You can stack up to four holding pens inside of each other if time allows.
- If you encountered any mortality, then retrieve the acoustic tag using the *Performing a Necropsy of Acoustically Tagged Chinook Salmon* SOP.
- Continue to release fish throughout the shift according to the schedule posted in the field crew trailer. At the end of your shift, make sure that the next shift of personnel arrives prior to leaving. The crew handling the last release will bring all supplies and equipment remaining at the release site and trailer back to the office.

References

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- Wedemeyer, G. 1996. *Physiology of fish in intensive culture systems*. New York, NY: Chapman & Hall, International Thompson Publishing.

Appendix E: Constructing and Deploying Trash-Can-Style Holding Pens

Standard Operating Procedure (SOP)

Adapted from the SOP prepared by Mike Marshall (U.S. Fish and Wildlife Service)

Purpose and Scope:

Acoustically tagged hatchery winter-run Chinook salmon and late-fall-run Chinook salmon from the 2015 Fremont Weir Fish Behavior Study have to be held for a minimum of 24 hr to acclimatize to the conditions of the Sacramento River before release. To accomplish this task, a holding pen setup crew for the study will have to construct and deploy trash can style holding pens at Jerry Rose's dock at Knights Landing. Deployment of the trash can style holding pens must occur before the transport crew arrives to Jerry Rose's dock with the salmon for release. Salmon should be placed into the holding pens on January 27, 2015, and February 2, 2015.

The SOP provides the steps that need to be taken to construct and deploy trash can style holding pens.

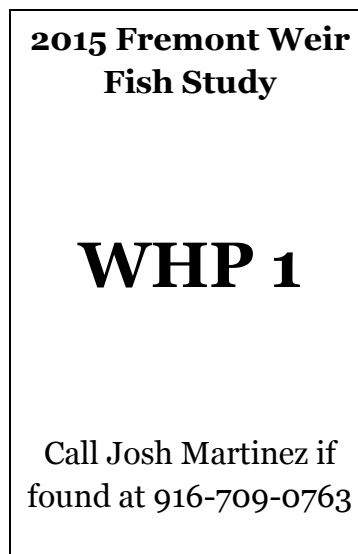
Materials (per pen):

1. Perforated 32-gallon Rubbermaid Brute trash can and lid
2. Float frames. If new ones need to be constructed, then the following materials are needed:
 - a. 3" diameter PVC pipe
 - b. 3" diameter t-fittings (4 per frame)
 - c. End cap (4 per frame)
 - d. PVC primer
 - e. PVC cement
3. 1/4x20x1" full-thread bolts (qty 4)
4. 1/4x20 washers (qty 4)
5. 1/4x20 nuts (qty 8)
6. 1/4x20 wing nuts (qty 4)
7. Heavy duty zip ties (qty 4, 18" minimum)
8. 1/4" to 1/2" rope (depending on flow)
9. Carabineers (qty 2)
10. Tools for constructing new perforated holding pens and float frames

- a. Putty knife
 - b. Sandpaper
 - c. Chop saw or saw with metal cutting blade
 - d. Reciprocating saw
 - e. Drill (cordless preferable, but not necessary)
 - f. 1/4" drill bits or smaller (depends on the size of fish)
11. Laminated label for the trash can/lid
 12. Duct tape for placing laminated label on the trash can/lid

Label Setup:

1. Print out and laminate 10 labels for the trash can lids, which include the information shown in the example below.



“WHP 1” stands for winter-run Chinook salmon holding pen #1. These labels are printed out from Microsoft Word. Each document should be labeled with either a “WHP” (winter-run holding pen) code or a “LFHP” (late-fall-run holding pen) code and numbered from one to five. These labels are 8.5” by 11”.

2. Print out the table below, cut out each individual square, and laminate. These labels are for the trash cans.

WHP 1	WHP 2	WHP 3	WHP 4	WHP 5
LFHP 1	LFHP 2	LFHP 3	LFHP 4	LFHP 5

Inspection and Construction:

1. Gather the “II” float frames to inspect equipment.
2. If not already present, attach one length of rope, approximately 2’ to 2.5’ long, to each of the pillars of the “II” float frame using a bowline knot. At the other end of each length of rope, attach a carabineer using a bowline knot. These ropes with carabineers will be used to attach the float frames to Jerry Rose’s dock. In the end, each float frame should look like the image below:



3. Inspect all of the float frames to make sure that they are functional and can float when placed in water. The following steps must be taken if more float frames need to be built:
 - a. Measure unused PVC pipe to the 19.5” mark and cut four lengths. Glue one of the openings of the t-fitting to one length of the PVC pipe. Continue gluing and connecting the straight pipes to the openings of the t-fitting until you create a square shape.
 - b. Once you have a square shape, measure the PVC pipe to the 10” mark and cut four lengths. Glue each remaining opening of the t-fitting to one length of the PVC pipe to create a “II” shape. Afterwards, close the PVC pipe by gluing on an end cap.

- i. Note: When gluing PVC, it is recommended that each connection is primed using PVC primer prior to gluing per manufacturer's recommendations.
4. Inspect all perforated trash cans to make sure the holes are appropriate for the size of fish that will be used for the study, and to make sure that there are no damages to the trash can that could affect its functionality. The minimum fork length of fish used for the study will be 90 mm. If the holes are not appropriate or if there are damages to the trash can, then the following steps must be performed to build new perforated trash cans:
 - a. Use a reciprocating saw to cut the lower part of the handle off, which will allow the float frame to set much higher on the can. As a result, this will increase the amount of water that is available for the fish.
 - i. Note: You need to make sure that there is an air/water interface so the fish can come up and "gulp" air.
 - b. Once you have removed the lower portion of the handle, you can begin drilling the holes in the sides and bottom of the holding container using the appropriate size drill bit. Drill holes from just below where the float frame attaches to all the way to the bottom.
 - i. Space the holes, in straight lines approximately 1" to 1.5" apart. Space the lines of holes approximately 1" to 1.5" apart.
 1. Note: Make sure that you are using a sharp, new drill bit. If you do not, then you will leave burrs on the interior of the container, which can harm the fish. If you do have burrs, use a flat edge or putty knife, along with sandpaper, to clean the burrs off.
5. Gather the lid to the trash can and place it on the trash can. The lid and the top of the can should have drilled holes. See if you can align the holes of the lid with the trash can. If the holes do not align or if there are no holes, then the following steps must be taken:
 - a. Attach the lid to the trash can and drill four holes at equal distances from each other, around the can. These holes should go through both the can and lid.
 - b. Mark each lid and orientation so in the future you will use the exact lid with the exact can so that all lids fit securely. As an example, you may want to letter side A and side B on both the can and the lid. This will assist with proper alignment in the future.
6. Once you are able to align the holes of the lid with the trash can, remove the lid and take a 1/4x20x1" bolt and thread a 1/4x20 nut up to the head of the bolt.
7. Fit the bolt through the hole in the trash can with the threads facing up. Once you have the bolt through the can, add a 1/4x20 washer and second nut. This will hold the hardware on the can so it will not fall out

- or move. Continue this process until you have all four holes fitted with hardware.
8. Once the trash can and lid are in place, duct tape a laminated 8.5" by 11" label with the holding pen number on the trash can lid. Afterwards, duct tape a laminated box label to the trash can itself. This allows you to identify which lid belongs to which trash can.
 9. To attach the frame to the trash can, use four heavy duty zip-ties and attach it using the holes in the trash can that are above where the float frame attaches (see red circle in image below). Make sure that the connection of the zip-tie is on the outside of the can.



- If the holes are not adequate or if no holes are present, complete the following tasks:
- a) Determine where you would like to attach the trash can to the frame by test fitting a frame to the trash can. Once you have determined this location, drill two vertical holes in the trash can, just above where the float frame attaches. In the end, there should be four paired holes.
 10. When holding containers are not in use, remove the float frames, lids, and hardware as damage to the holding containers will occur and storage will be problematic.

Deployment:

1. Before heading to Jerry Rose's dock during the day of deployment, gather all the necessary materials:
 - a. Perforated trash cans with lids and mounting hardware attached
 - b. Float frames
 - c. 1/4x20 wing nuts (4 per trash can, keep spares on-hand)
 - d. Heavy duty zip ties (18" minimum)
 - e. Field notebook for taking notes
 - f. YSI ProODO (will be used to measure water temperature and dissolved oxygen the site)
 - g. PFDs
 - h. Headlamps, lanterns, and flashlights (to set up in field crew trailer)
 - i. First aid kit (to set up in field crew trailer)
 - j. Atomic clock (to set up in field crew trailer)
 - k. Throw ropes (to set up in field crew trailer and attached to the dock)
 - l. DWR pool phone (to set up in field crew trailer)
2. Upon arriving at Jerry Rose's dock, the holding pens will be deployed on the side of the dock closest to shore (see picture below).



3. Evaluate the water levels of the area where you will deploy the holding pens. If water levels are too low, then plan on deploying the holding pens on the other side of the dock where the water is deeper.
4. Once you have identified the area for deployment, create loops in the rope at approximately 24" to 30" apart. You will use these loops to attach the carabineer from the float frame (attached to the holding container) to the rope.

5. Connect your rope line with the loops by using the cleats on the dock. As you do this, pull the rest of your rope downstream to make the rope tight.
 - a. Note: Make sure that you have an adequate number of loops for the number of cans you have.
6. To attach the float frames to the holding pens, use heavy-duty zip ties based on the process described in the “Inspection and Construction” section of the SOP.
7. To attach the lids to the trash cans, follow steps 7 and 8 in the “Inspection and Construction” section of the SOP.
8. To attach the float frame with trash can, clip the carabineer to the first loop on the rope line. Afterwards, clip the second carabineer of the frame to the second loop. The next can’s first carabineer should be clipped to the second loop. This means that the second loop will have two carabineers.
 - a. Follow this method until all of the holding pens are attached to the rope line. In the end, the holding pens should be set up in chronological order by species:

WHP 1	LFHP 1	WHP 2	LFHP 2	WHP 3	LFHP 3	WHP 4	LFHP 4	WHP 5	LFHP 5
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9. As the setup crew is working to deploy the holding pens, a transport crewmember will call a release crewmember and will provide the water temperatures in the coolers containing fish at the water quality check station near Granzella's Restaurant in Williams, California. This is done so the holding pen setup crew can assess the need for tempering the fish at the release site. The holding pen setup crew should have a YSI ProODO meter with them during the deployment process.
10. As the setup crew is deploying the holding pens, the trailer and portable toilet should arrive at the release site. The setup crew should provide access to staff transporting the trailer and portable toilet.
11. Upon assembling the holding pens, the headlamps, lanterns, flashlights, atomic clock, first aid kit, throw ropes, and a YSI ProODO should be placed into the trailer. The release crew will be responsible for cleaning up after all the fish releases have occurred.

Appendix F: Performing a Necropsy of Acoustically Tagged Chinook Salmon

Standard Operating Procedure (SOP)

Adapted from the SOPs used for the Department of Interior's south Delta telemetric studies.

Purpose and Scope:

Mortality of tagged hatchery Chinook salmon could occur during the tagging, fish care, transport, or release process during the 2015 Fremont Weir Fish Behavior Study. The dead salmon (mort) should be bagged in a Ziploc bag and labeled with the date, the time and the location of bagging (e.g., circular ID or holding pen ID). The only time the study crew would not bag a mort is when the study crew notices the mortality in the recovery bucket since the tag can be reused after the necropsy. Specific instructions on how to handle the morts are described in the following SOPs for the 2015 Fremont Weir Fish Behavior Study:

1. SOP #1a: Acoustically Tagging Winter-run Chinook Salmon at the Livingston Stone National Fish Hatchery,
2. SOP #1b: Acoustically Tagging Late Fall-run Chinook Salmon at the Coleman National Fish Hatchery,
3. SOP #3: Daily Fish Care after Tagging at the Livingston Stone National Fish Hatchery, and
4. SOP #4: Transporting, Holding and Releasing Acoustically Tagged Winter-run Chinook Salmon.

Once the mort has been bagged or is in a recovery bucket, the study crew must evaluate the conditions of the fish, perform a necropsy, and retrieve the acoustic tag. This SOP describes the steps that need to be taken for performing these tasks.

Points of Contact:

Any reference to the tagging coordinator in this SOP refers to the following staff. Please contact the lead contact before the back up.

Role	Name	E-mail	Office Phone	Cell Phone
Lead Contact	Josh Israel	jaisrael@usbr.gov	916-414-2417	916-296-8792
Back Up	Jason Hassrick	jhassrick@usbr.gov	916-414-2416	916-425-9121

Materials:

1. *Hatchery Fish Condition Assessment Datasheet* (used during the tagging and fish care process; one for each hatchery)
2. *Hatchery Acoustic Tag Envelope* (used during the tagging and fish care process; one for each hatchery)
3. Hatchery designated camera
4. *Transport/Release Fish Condition Assessment Datasheet* (used during the transport and release process; one for each species)
5. *Transport/Release Acoustic Tag Envelope* (used during the transport and release process; one for each species)
6. Transport/release designated camera
7. Forceps
8. Tray
9. Dissecting probe
10. Scalpel handle, holder and blades
11. Ziploc bags and Sharpie marker
12. Nitrile gloves
13. VR100 with 180 VH hydrophone (only needed for the tagging coordinator)

Items 1 to 3 will remain at the hatchery until there are no more study fish.

Procedures:

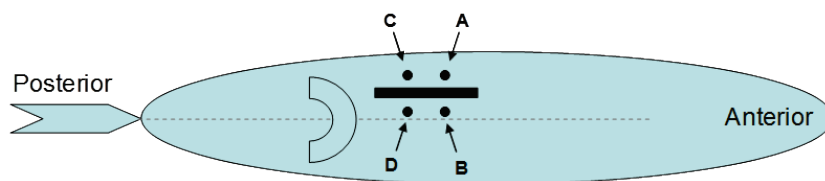
- G. Put on gloves when handling fish.
- H. Remove the salmon from the Ziploc bag. If the mortality occurred in a recovery bucket, then remove the salmon from the recovery bucket instead of a Ziploc bag.

- I. Label the Ziploc bag using a Sharpie marker with an unused fish ID (e.g., label it as “Fish ID # W1”) from the hatchery or the transport/release *Fish Condition Assessment Datasheet*. The fish ID is used to identify the salmon until the tag code ID is known.
 - a. If the mortality was from a recovery bucket, then write the fish ID on a piece of paper.
- J. Take a picture of the Ziploc bag with the fish ID. Make sure the camera date and time stamp are on. This picture is used to identify the number of pictures taken per fish.
 1. If the mortality was from a recovery bucket, then take a picture of the piece of paper with the fish ID. Afterwards, the piece of paper can be recycled.
- K. Afterwards, take a picture of the salmon showing the sutures.
 1. At a minimum, there should be two pictures per fish ID: one of the Ziploc bag and one of the sutures. However, the study crew should take as many pictures as they feel are necessary. In the end, the study crew needs to keep track of how many pictures are taken per fish ID. This should be recorded in the hatchery or transport/release *Fish Condition Assessment Datasheet*.
- L. Fill out the general section of the *Fish Condition Assessment Datasheet*.
 1. Examples of how to record the location of bagging is below:
 - i. CIR 1= Mortality found in circular #1 at the hatchery
 - ii. WC 1= Mortality found in cooler #1 of winter-run Chinook salmon
 - iii. WHP 1= Mortality found in holding pen #1 for winter-run Chinook salmon
 - iv. RB= Mortality found in the recovery bucket. There is no need for a numeric designation after the RB code since recovery buckets are reused during the tagging process (see SOP #1a/1b).
- M. Check the five characteristics of condition (scale condition, body color, gill color, eye condition, and fin hemorrhaging) and record the information on the *Fish Condition Assessment Datasheet*:
 1. Scale: Determine whether there is any descaling. Scale condition is noted as “N” (Normal), “P” (Partial), or “D” (Descaled) and is assessed on the most compromised side of each fish. The codes are defined as follows:
 - i. N= Loss of less than 5% of the scales on one side of the fish
 - ii. P= Loss of 6 to 19% of the scales on one side of the fish

- iii. D= Loss of 20% or more of the scales on one side of the fish
- 2. Body Color: Determine the color on the dorsal side of the salmon using the following codes:
 - i. G= Dark pigmentation and good contrast
 - ii. B= Lighter or faded pigmentation and weak contrast
- 3. Gill Color: Lift the operculum using forceps and rank the darkness of the gills using the following codes:
 - i. G= Beet red to dark cherry red
 - ii. B= Lighter red to grayish/whitish color
- 4. Eyes: Determine if the eyes appear normally shaped or are bulging. To record the information, use the following codes:
 - i. G= Normal appearance
 - ii. B= Abnormal appearance and some bulging seen
- 5. Fin Hemorrhaging: Determine if there are spots of blood on or at the base of the fins. To record the information, use the following codes:
 - i. G= No hemorrhaging seen
 - ii. B= Hemorrhaging seen

N. Evaluate and record the conditions of the suture on the *Fish Condition Assessment Datasheet*:

- 1. Suture Present: Evaluate whether the suture is present on both the anterior and posterior side.
 - i. Record the information in fractional form (i.e., anterior/posterior).
 - ii. Use 1 if the suture is present and 0 if the suture is not present.
 - 1. Example: A “1/1” code indicates that both the anterior and posterior suture are present.
- 2. Irritation: Determine if there is irritation present at any suture site.
 - i. Use A, B, C, D code in the diagram below to refer to the suture site:



View looking down onto incision; suture entry and exit points

- ii. Use the following codes to evaluate suture condition:
 - 1. 0= No irritation
 - 2. 1= Mild irritation (redness or swelling)
 - 3. 2= Moderate irritation (redness or swelling)

4. 3= Severe irritation (purulent discharge)
 5. 4= Ulceration
 3. Incision Apposition: Record “Y” if completely closed or “N” if not completely closed.
 4. Incision Healing: Record “Y” if completely healed or “N” if not completely healed.
 5. Fungus: Record “Y” if fungus is growing around the suture or “N” if no fungus is present.
 6. Tag Expulsion: Record “Y” if there is tag expulsion or “N” if there is no tag expulsion.
- O. Using a scalpel blade and dissecting probe, cut open the incision area of the fish and note the location of the tag in the fish. Record the location of the tag in the “Tag Location” column using the following codes:
1. 0= Tag is directly under the incision.
 2. 1= Tag has shifted toward the anterior side of the fish.
 3. 2= Tag has shifted toward the posterior side of the fish.
- P. Describe any organ damage that may have occurred from the acoustic tag or if there are any indications of disease in the comments section on page 2 of the hatchery or transport/release *Fish Condition Assessment Datasheet*.
- Q. Retrieve the acoustic tag from the fish and place the tag back in the Ziploc bag that was used to hold the fish. The carcass can be tossed into the river if the mortality occurred at the river site. If the mortality occurred at the hatchery, then dispose the carcass in the dumpster at the hatchery
1. Note that the use of a Ziploc bag in Steps 11 and 12 are not applicable if the mortality occurred in the recovery bucket and the acoustic tag can be reused again.
- R. Place the Ziploc bag with the acoustic tag in the *Acoustic Tag Envelope*. The *Acoustic Tag Envelope* is used to store all retrieved acoustic tags for each species at a given location.
1. The transport crew is responsible for picking up the **hatchery** *Acoustic Tag Envelope* and the **hatchery** *Fish Condition Assessment Datasheet* from the fish care crew on the last transport day. Afterwards, the transport crew will bring back the materials to the tagging coordinator.
 2. The release crew for the last release of each week is responsible for returning the **transport/release** *Acoustic Tag Envelope(s)*, the **transport/release** *Fish Condition Assessment Datasheet(s)*, and other materials back to the tagging coordinator.

- S. Once the *Acoustic Tag Envelope(s)* are back with the tagging coordinator, the tagging coordinator will identify the tag code ID of each tag using a VR-100 with a 180 VH hydrophone. Afterwards, the tagging coordinator will record the information on the hatchery or transport/release *Fish Condition Assessment Datasheet(s)*.

Hatchery Fish Condition Assessment Datasheet

2015 Fremont Weir Fish Behavior Study

[illegible]

Hatchery Fish Condition Assessment Datasheet (cont)

2015 Fremont Weir Fish Behavior Study

Fish ID	Comments	Tag Code ID
Ex	Kidney is damaged, ribs are broken, and the incision is too far from the midline. Organs look inflamed.	
W1		
W2		
W3		
W4		
W5		
W6		
W7		
W8		

Transport/Release Fish Condition Assessment Datasheet

2015 Fremont Weir Fish Behavior Study

[illegible]

Transport/Release Fish Condition Assessment Datasheet (cont)

2015 Fremont Weir Fish Behavior Study

Fish ID	Comments	Tag Code ID
Ex	Kidney is damaged, ribs are broken, and the incision is too far from the midline. Organs look inflamed.	
W19		
W20		
W21		
W22		
W23		
W24		
W25		
W26		

Appendix G: ADCP Data Collection for 3D Computational Fluid Dynamic Modeling

Standard Operating Procedure (SOP)

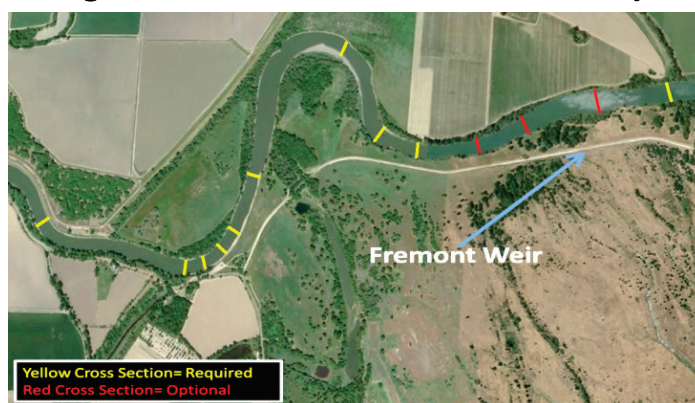
Adapted from the protocols written by Paul Stumpner of the US Geological Survey.

Purpose and Scope:

The primary purpose of this SOP is to outline the expectations and guidelines for the ADCP surveys that will be performed by the California Department of Water Resources (DWR) for the 2015 Fremont Weir Fish Behavior Study. Each survey will require three crewmembers. At a minimum, DWR will conduct two to four surveys under different flow conditions at 10 cross sections (see yellow cross sections in Figure 1). Each cross section should have six passes in order to measure secondary circulation. Results from these surveys will be used to verify results from the computational fluid dynamic modeling for the 2015 Fremont Weir Fish Behavior Study.

However, there is flexibility to adjust the location of the cross sections if field conditions warrant a change. If there is a change, then this must be documented for the Fremont Weir Study Coordination Team. Moreover, the study crew can survey more cross sections if time permits on a given day. In particular, the study crew could survey the three red-cross sections in Figure G1.

Figure G1: Cross sections for each ADCP survey.



The first survey will occur before the first fish release into the Sacramento River, which is expected to occur on January 28, 2015. To avoid data collection that could influence the reception of the acoustic receivers or juvenile swimming behavior, ADCP surveys should not be collected within three to five days of a fish release.

The remaining three surveys will be performed under different flow conditions than the first survey and will be scheduled based on the recommendations from the coordination team. There is a possibility that four surveys may not be needed.

Field Methods:

1. Equipment Setup

- Velocity mapping will be conducted with a RDI Rio Grande Workhorse acoustic Doppler current profiler (ADCP) and a differential GPS. The ADCP and GPS will be mounted on the side of a manned boat with the GPS located directly over the ADCP to ensure geo-referencing consistency, and high enough to avoid multipath errors to the extent possible. The ADCP mount needs to be guyed down to the stern and bow of the boat to prevent excessive pitch. The ADCP baud rate should be set at 38400. The GPS should be set up to output data at 4 or 5 Hz, and a baud rate of 19200 or higher.

2. Weather Conditions

- Weather conditions at the start of each day must be recorded and changes in weather conditions should be documented with the time of day for each survey date.

3. WinRiver II Data Collection Setup

- WinRiver II is the data collection software used for the ADCP and GPS. A user's guide is available for more in-depth information about the principles of operation and setup. Below are a few general remarks on the specific information that is needed.
- Make sure units are set to SI.
- Use the configuration wizard to create a new measurement.
- The max water depth, water speed, and boat speed should be set at reasonable high values, but not too high (i.e., if you expect the max depth to be 10m, set the max depth at 12 or 13m). A preliminary transect can be taken to refine these values if necessary.
- Use water mode 1 (WM1) and bottom mode 5 (BM5).
- Enter the correct transducer depth and magnetic variation.
- The configuration wizard will set the bin size (WS) and number of bins (WN). The team want 25 cm bins (WS25). If the WS command is changed, then WN needs to be changed inversely

proportional to reflect this change. For example, a change from WS50 to WS25 would result in a change of WN10 to WN20.

- If the wizard does not allow for WM1 and BM5 to be set, then these need to be entered in the user commands.
- Lastly, the following additional user commands should be entered: water pings 3 (WP3) and bottom pings 2 (BP2). This does some initial low-level averaging.

4. Data Collection

- Cross-section locations should be determined beforehand. If possible, a navigational software should be used to ensure boat course consistency. The same practices as those used for the moving boat discharge measurements should be made for velocity mapping. Nonetheless, the following are basic guidelines for data collection.
- First, perform pre-data collection tests (i.e., ADCP test and compass calibration). You should first calibrate the compass. Ideally, the total error should be less than 1°, but 2° is acceptable. An evaluation should be done after calibration.
- The cross section should be normal to the flow direction.
- Six repeated transects for each cross section of the river should be collected. Four transects may be sufficient to analyze secondary circulation. However, if there are GPS errors, boat course errors or any other errors, then the transects can be sub-sectioned or disregarded.
- The repeated transects should be as close to one another as possible.
- For each cross section, a separate file should be generated (i.e., 6 files for each cross-section). At the start and end of each transect (or file), the edge estimates should be inputted.

Processing Methods:

Ideally, the files can be preliminarily processed in the field to determine whether the data has been collected correctly. After all data has been collected, the final processing can occur at the office.

1. Initial Processing in WinRiver II

- Perform any necessary processing (i.e., corrections to transducer depth or magnetic variation) or sub-sectioning of data in WinRiver II.
- Make sure units are in SI.
- Once all files have been processed, output ASCII files under Configure → ASCII Output → Classic ASCII Output. Choose Output Backscatter Intensity.

- Now reprocess all transects under Playback → Reprocessed Checked Transects. This will create files with the _ASC.txt extension.

2. Processing in Velocity Mapping Toolbox (VMT)

- Each cross-section needs to be processed separately. Open VMT and then open ASCII files; select all files for a particular cross section.
- To process the data, you need to choose Plot Cross Section; prior to that, the following parameters should be set.
 Grid Node Spacing: Horz – 1, Vert – 0.25
 Contour Variable: Streamwise Velocity (u)
 Vertical Exaggeration: 2 or 3
 Vector Scale: 0.2
 Vector Spacing: Horz – 4, Vert – 0.25
 Smoothing Window: Horz – 7, Vert – 5
 Check the box: Plot Secondary Flow Vectors
 Secondary Flow Variable: Secondary (Roz)
 Check the box: Include Vertical Velocity Component in Sec-

ondary Flow

Vector

- The Vertical Exaggeration and Vector Spacing can be changed for plotting purposes, and these will probably be cross-section specific. The Grid Node Spacing and Smoothing Windows should remain consistent for each cross section; changes to these will change the final results.
- Save the log file.
- Save the MAT file.

3. Export Final Data Products

- Run the MATLAB script. WriteVMTOutputToCSV.m. This can be done in batch mode and will output seven files for each cross section. The following extensions with the filename as a prefix will be created:

‘Filename_Timerange.csv’ – time range that data was collected

‘Filename _Easting.csv’ – N x M array of UTM Eastings for each cross-section

‘Filename _Northing.csv’ – N x M array of UTM Northings for each cross-section

‘Filename _Depth.csv’ – N x M array of depth below water surface for each cross-section

‘Filename _U.csv’ – N x M array of U component of velocity for each cross-section

'Filename _V.csv' – N x M array of V component of velocity for each cross-section

'Filename _W.csv' – N x M array of W component of velocity for each cross-section

Format of Deliverable:

DWR will compile the dataset for delivery to the Fremont Weir Study Coordination Team and will be available to draft up descriptions about the results and field measurements. In addition, any weather conditions and field observations that could have affected the data should be reported. This includes adding additional cross sections or adjusting the location of the proposed cross sections.

The coordinate system provided to the coordination team should be in the same format that was used for the bathymetric deliverables.

Appendix H: Bathymetry for 3D Computational Fluid Dynamic Modeling

Standard Operating Procedure (SOP)

Adapted from the field descriptions by Jim West of the California Department of Water Resources.

Memorandum

Date: April 2015

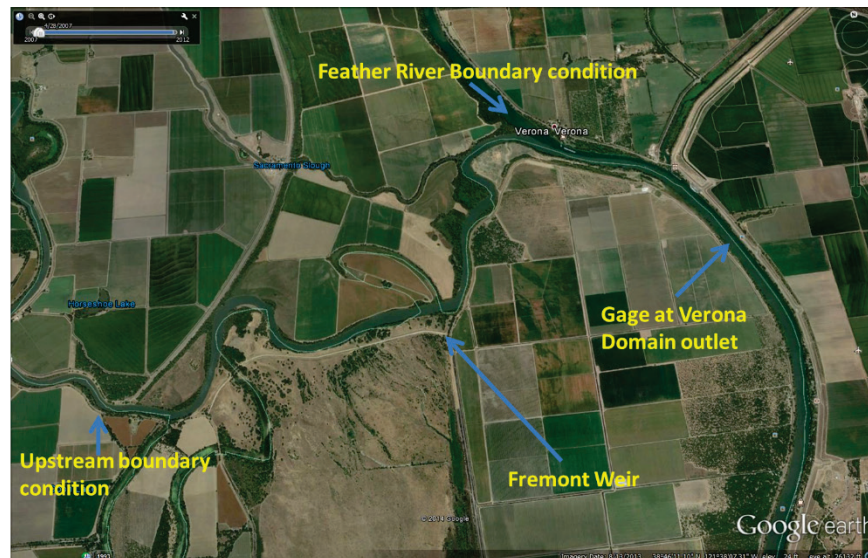
To: Edmund Yu
Dept of Water Resources, Division of Environmental Services

From: Jim West
Dept of Water Resources,
Division of Integrated Regional Water Management

Subject: Field Memo on Collecting Bathymetry for the 2015 Fremont Weir Fish Behavior Study

From January 21, 2015, to January 27, 2015, my field crew collected bathymetry on the Sacramento River, near the Fremont Weir area. Our survey covered three boundaries: the upstream boundary condition, the Feather River boundary condition, and the gage at the Verona domain outlet (see Figure 1). Although the team were able to complete the survey, my crew encountered numerous problems during the survey, such as laptop issues and the radio failure of the Global Navigation Satellite System (GNSS) equipment that the team was using to collect Global Positioning System (GPS) data. Because of this, the team had to rent equipment and make changes to the standard operating procedure (SOP) that was developed for the survey.

Figure H1: Boundaries for the bathymetric survey.



The changes made to the SOP is documented at the end of this memorandum in tracked changes. A few of the key changes are described below.

- For the first part of the survey, the team had three Trimble R8 GNSS receivers. One served as the primary control point, while the other two served as the rovers in the boat. Due to a hardware failure in the radio component of one of the R8 receivers, the Trimble R10 receivers had to be used for the second part of the survey. Even so, the performance and accuracy of these receivers is nearly identical to that of the R8.
- For the bathymetry soundings, the team was planning to use the SonTek M9 with HydroSurveyor firmware. However, the team had to make a change to the Knudsen Engineering Limited Sounder 1612 survey-grade echosounder and transducer due to equipment issues with the SonTek M9. The change in field gear resulted in primarily cross sectional sweeps without longitudinal profiles, which were needed to inform the Yolo Bypass Salmonid Habitat Restoration and Fish Passage Project. As a result, the team went back out on April 8, 2015, to collect longitudinal profiles at the center line, left bank, and right bank of the river (see Figure 2).

Figure H2: Data collection points from surveys.



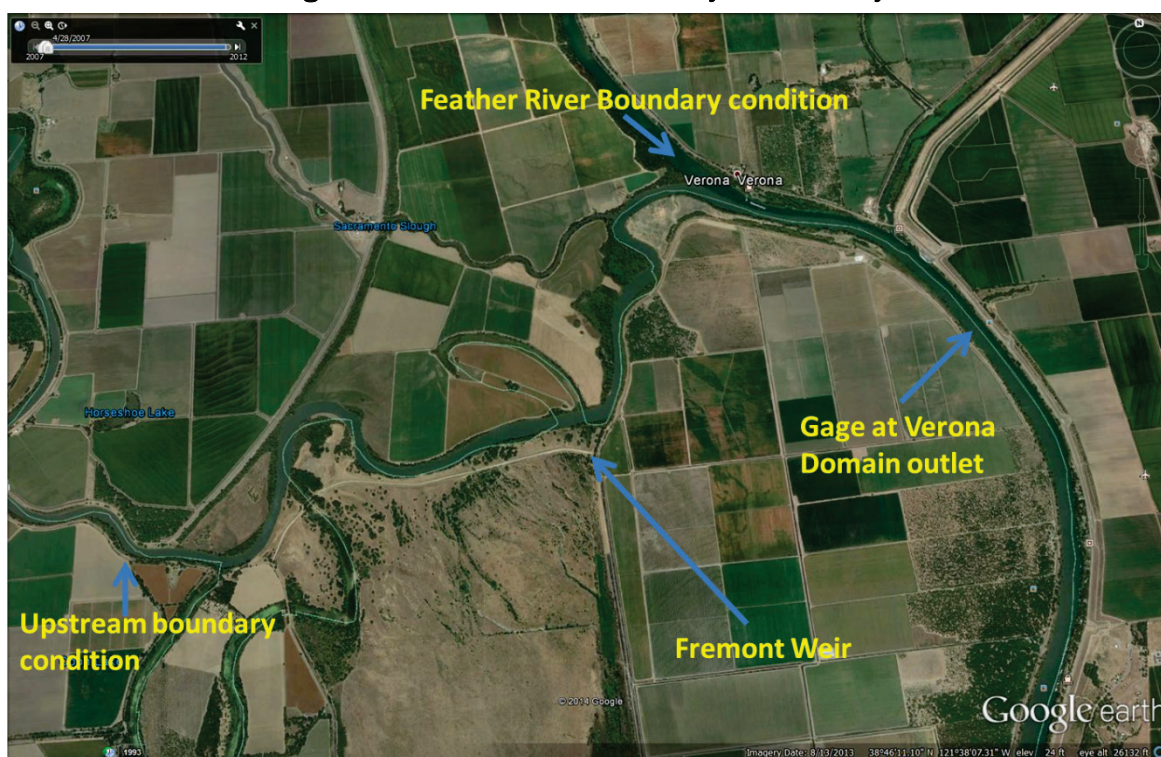
All data collected from our surveys have been processed and transmitted to the appropriate staff for their analyses. If you have any questions, please let Jim West, California Department of Water Resources (DWR), know.

Purpose and Scope:

The primary purpose of this SOP is to outline the expectations and guidelines for the bathymetric survey that will be performed by the DWR during the week of January 19, 2015. During the survey, bathymetric measurements will be taken throughout the project domain. This domain covers three boundaries: the upstream boundary condition, the Feather River boundary condition, and the gage at Verona domain outlet (see Figure 1). A single survey will take approximately one week to conduct and will require three crewmembers.

Results from this survey will be used for computational fluid dynamic modeling for the 2015 Fremont Weir Fish Behavior Study.

Figure H3. Boundaries for the bathymetric survey.



Field Methods

A. GPS Data Collection

DWR will use the Global Navigation Satellite System (GNSS) survey instruments for the survey(s). There will be a Trimble R8 GNSS receiver at the primary control point. In addition, there

will be two Trimble R8 GNSS receivers that will serve as the rovers in the boat. These receivers have 220 channels and can track signals from both the United States Global Positioning System (GPS) and the Russian GLObal Navigation Satellite System (GLONASS) satellites.

Due to a hardware failure in the radio component of one of the R8 receivers, Trimble R10 receivers had to be used for the second part of the survey. These receivers are also GNSS receivers with 440 channels. The performance and accuracy of these receivers is nearly identical to that of the R8. Datasheets for both instruments are provided for reference.

All of these receivers (R8 and R10) are survey-grade and are dual-frequency. These receivers observe carrier phase satellite measurements on both the L1 and L2 frequencies for both GNSS systems. Moreover, they can compute a position using a combination of satellites from both systems. As such, these receivers will provide centimeter level accuracy in both the horizontal and vertical positioning. For all RTK points, the GNSS elevations (ellipsoid heights) will be reduced to orthometric heights (ground elevations) using Geoid09. GNSS data processing will be done using the Trimble Business Center software.

B. Bathymetric Soundings

Equipment used for acquiring the bathymetric soundings consisted of a Knudsen Engineering Limited Sounder 1612 survey-grade echosounder and transducer. This type of echosounder is an acoustic echo ranging device; the depths are calculated by measuring the time it takes for a pulse of ultrasound to be transmitted downward from the transducer, to be reflected off the bottom, and to be returned to the transducer. Several factors affect the accuracy of soundings, including the following: bottom characteristics, depth, pulse length used, applied speed of sound through water (which is affected by clarity, salinity, and temperature), and field techniques. The field procedures and techniques used for this bathymetric project were designed to provide a high level of precision and have been used on several previous projects with good results. These procedures included,

but were not limited to, the following: the use of suitable control, the daily check and calibration of the echosounder and transducer, and the use of survey-grade GNSS receivers to provide centimeter-level positioning.

The GPS and bathymetric data were compiled in real-time using Coastal Oceanographic's Hypack software. This program uses the time stamps from the computer, GPS, and echosounder to correlate the data. The GPS antenna was placed directly over the transducer to greatly reduce the errors that can occur during turns and vessel heave. Soundings were measured every 50 msec. By maintaining a speed of less than four miles per hour, this procedure resulted in approximately twenty measurements per six feet of horizontal movement.

To convert the sounding data to the NAVD88 vertical datum, GNSS elevations (ellipsoid heights) will be reduced to orthometric heights (ground elevations) with the use of Geoid09.

C. Bar Checks

DWR will check and calibrate the bathymetric equipment on a daily basis by performing a bar check. The process of the bar check involves adjusting the draft to yield the correct depth to a known point in shallow water. Afterwards, the echo return from a deeper point of known depth is measured and the speed of sound in water is adjusted until the proper depth is obtained.

D. Weather Conditions

Weather conditions at the start of each day must be recorded and changes in weather conditions should be documented with the time of day for each survey date.

Data Processing and Format of Deliverable

DWR will decide on the appropriate software for compiling the GNSS and bathymetric sounding data after the survey. Soundings will be measured every few msec, which will result in more data points than what may be necessary. To help with data interpretation, DWR will work on thinning

the data, which would involve removing false soundings and erroneous data.

Afterwards, DWR will compile the dataset into an X, Y, and Z file for delivery to the Fremont Weir Study Coordination Team and will be available to draft up descriptions about the results and field measurements. In addition, any weather conditions and field observations that could have affected the data should be reported.

The delivered data should be referenced horizontally to NAD83, CCS83, State Plane Zone 2, and the vertical datum is NAVD88. All units should be provided in meters.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
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			5b. GRANT NUMBER N/A		
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14. ABSTRACT To improve modeling of juvenile salmon behavior and movement in the Sacramento River, smaller winter-run Chinook and larger late-fall-run Chinook salmon were tagged and released into a 2D telemetry array during the winter of 2015. Detection positions were filtered and discretized to create two-dimensional tracks and measure movement characteristics, evaluate space use, and assess whether these runs displayed distinct behavioral differences. Speed over ground and turning angle were not significantly different between release times, fish size, or run. Only the initial movement rate between release and array locations was significantly different between the runs. Both runs displayed a non-uniform distribution within the channel and tended to use space along the outer bend more frequently than the inner bend. Winter-run Chinook salmon tracks were slightly farther towards the outer bend than late-fall-run Chinook. A similar result was not observed in smaller and larger late-fall-run Chinook, which suggested that differential space use may be influenced more by run identity than variation in size between runs. Although small differences between runs were measured, it is reasonable to aggregate these results for a singular juvenile salmon behavior model, rather than developing independent juvenile behavior models based on adult run-timing.					
15. SUBJECT TERMS Salmon behavior, winter-run Chinook, late-fall-run Chinook, juvenile salmon behavior models					
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